The genetic consequences of demography and disturbance in small mammal populations

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Declaration

This thesis is my original work. I am the senior author of all chapters. While all chapters were the product of collaborations carried out jointly with others, I am the principle contributor to the work. This thesis has not been submitted for any other degree or diploma in any university. This research was carried out under approval from the Australian National University Ethics Committee (Protocol A2014/23).

Robyn E. Shaw

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Thesis Plan

This thesis is presented as six chapters, including a general introduction and concluding remarks. Figures, tables, references and appendices can be found at the end of each chapter. Chapter 2 has been published, while the remaining data chapters are presented as manuscripts intended for submission. For this reason, I use the pronoun "we" to represent coauthors in the published chapter and the chapters intended for submission. However, in all chapters, I designed the research, conducted the field work, performed the statistical analyses and authored the chapters. Below, I provide details on the specific contributions made by my coauthors.

Chapter 2: The impact of mating systems and dispersal on fine-scale genetic structure at maternally, paternally and biparentally inherited markers.

I designed the study (in collaboration with Sam Banks and Rod Peakall), ran simulations and downstream analyses and authored the chapter. Sam Banks provided organismal expertise for the agile antechinus, Rod Peakall completed the simulation programming, and all authors contributed to editing this chapter.

Chapter 3: Evaluating population genetic patterns across molecular markers, alignment methods and SNP filtering strategies in a native Australian rodent

This work was carried out in collaboration with the Australian Wildlife Conservancy (AWC). I collected the samples in the field, carried out the DNA extractions (sent to Diversity Arrays Technology™ for genotyping), performed the mitochondrial and microsatellite sequencing, carried out the bioinformatics and statistical analysis and authored the chapter. Sam Banks and Rod Peakall contributed to the design of the study and provided comments on the chapter. Cameron Jack carried out the reference-based alignment.

Chapter 4: Habitat preferences, fire response and recovery in an Australian native rodent

This work was carried out in collaboration with AWC. I carried out the field work, designed the study, performed the statistical analyses and authored the chapter. Rod Peakall and Geoff Cary contributed to the study design and provided comments on the chapter. Sam

Banks, Sarah Legge, Katherine Tuft and Alex James contributed to the conceptual development of the work, planning and implementing the fire experiment (along with help from Hugh McGregor and Toby Barton), and provided comments on a draft.

Chapter 5: Genetic evidence suggests mechanisms for post-fire recovery differ with the extent of experimental fire

This work was carried out in collaboration with AWC. I designed the study, carried out the field work, performed the DNA extractions (later sent to Diversity Arrays Technology™ for genotyping), performed the statistical analyses and authored the chapter. Rod Peakall, Sam Banks, Sarah Legge, Katherine Tuft and Alex James contributed to the conceptual development of the work and planning and implementing the fire experiment (along with help from Hugh McGregor and Toby Barton). Sam Banks, Rod Peakall and Alex James all provided comments on the chapter.

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Lastly, I'd like to thank Jordan. You've kept me sane, and your support and love has been everything.

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Abstract

Social, demographic and ecological processes shape patterns of genetic diversity. These patterns can therefore reveal insights into the biology of species and the response of populations to disturbance. During my PhD, I used a combination of computer simulations, molecular techniques and field-based experiments to explore how biological and ecological processes shape populations and their underlying genetic diversity.

Dispersal and mating systems have long been known to shape population-level patterns of genetic structure. However, few studies focus on how these processes shape spatial genetic patterns within populations. Using the agile antechinus (*Antechinus agilis*) as a model, I carried out computer simulations to investigate how dispersal and mating behaviour shape fine-scale genetic structure (over the scale of metres) across autosomal, mitochondrial and Y chromosome markers. While dispersal was the major driver of fine-scale genetic structure, variation in mating behaviour also created differences in the level of structure detected at uniparentally inherited markers. Thus, comparing sex-specific patterns across markers with differing modes of inheritance can help elucidate demographic processes occurring within populations.

In addition to microsatellite, mitochondrial and Y chromosome markers, high throughput sequencing data is becoming increasingly accessible for ecological research. However, decisions about marker choice, bioinformatic pipelines and filtering can be overwhelming for experts and non-experts alike. Through my empirical research focusing on a native Australian rodent, the pale field-rat (*Rattus tunneyi*), in the Kimberley region of Western Australia, I explored how marker choice and bioinformatic methods influence biological conclusions. Genetic analyses revealed low levels of genetic structure across this disturbance-prone landscape. While population-level estimates of genetic structure were fairly robust, measures of heterozygosity and diversity differed among marker types and filtering criteria. This demonstrates the importance of understanding how methodological decisions can impact biological inference from genetic data.

The pale field-rat is one of many small mammals declining across northern Australia. This is due, in part, to the interaction between altered fire regimes and other

key threats. To better understand this decline, I investigated habitat preferences, fire response and post-fire population recovery using a replicated fire experiment and capture-mark-recapture study. Mixed modelling showed that capture rate was negatively correlated with the extent of experimental fire, and that pale field-rat habitat preferences did not change in the post-fire landscape. However, all populations completely recovered one year after fire.

The fire experiment suggested that spatial recovery processes differed according to the size and spatial pattern of fires. To test these different recovery hypotheses, I used parentage and genetic spatial autocorrelation analysis to explore patterns of relatedness before and after fire. This indicated that post-fire recovery after patchy fires was driven by *in situ* survivors from within unburnt refuges, compared to recolonisation after thorough fires. Furthermore, changes in female dispersal strategies appeared to be driving these different recovery patterns. These results suggest that fire management should aim to maximise the patchiness of burns and limit their extent in order to facilitate recovery of small mammals in this system.

My thesis demonstrates that the combined use of computer simulations, direct field research and genetic analyses can reveal novel insights into the demographic processes occurring within populations and the response of populations to disturbance. I discuss how these insights add to our understanding of mammal declines in northern Australia and can be used to inform fire management.

Chapter 1

General Introduction

Dispersal and reproduction are fundamental life history traits that can have important evolutionary and ecological consequences for populations and species. These processes and their interaction are the basis of social systems and influence the spatial distribution of genotypes across the landscape (Greenwood 1980, Chesser 1991, Sugg et al. 1996). Thus, understanding these processes has been central to ecological, evolutionary and population genetics research. Knowledge of dispersal and reproductive strategies can help us to predict how species may adapt to changing conditions (Lenormand 2002, Kokko and López-Sepulcre 2006, Schradin et al. 2012), plan conservation strategies for vulnerable species (Hanski and Thomas 1994, Steiner et al. 2013), and further ecological and evolutionary theory. Furthermore, understanding these processes can also provide insight into how animal populations respond to ecological disturbance, which is of critical importance in the face of global change (Shea et al. 2004, Davies et al. 2016).

During my PhD, my research has focused on these two important processes and how they influence patterns of genetic structure in animal populations. This interest has also lead me to explore questions about how small mammal populations respond to ecological disturbance, and in particular the role of dispersal in population recovery following fire. I use a simulation study to explore how spatial genetic patterns respond to variation in dispersal and mating systems across a range of different molecular marker types. I also investigate how different marker types and bioinformatic processing influence the outcomes of downstream genetic analyses, in order to develop a high confidence panel of markers for use in a later chapter. I then focus on a case study, investigating demographic and genetic responses of a native rodent to fire in north-western Australia, conducting a fire experiment and using ecological and genetic information to understand how native mammals respond to fire. This research is linked with a larger fire management research program led by the Australian Wildlife Conservancy. Below, I provide a brief introduction to the concepts I investigate in my thesis.

Demography and disturbance

There is an incredible diversity of dispersal and reproductive strategies in animal populations (McEachern et al. 2009, Blyton et al. 2012). Dispersal, the movement of

individuals from where they are born to where they reproduce, can range from natal philopatry through to high dispersal (Duputié and Massol 2013). Dispersal strategies can vary in response to environmental or demographic stochasticity, and can differ between the sexes (sex-biased dispersal) or among individuals (dispersal polymorphism) (Lawson Handley and Perrin 2007, Bonte et al. 2010). Mating systems also vary along a continuum, from complete monogamy through to promiscuity. While much variation exists, male-biased dispersal is often associated with a polygynous mating system, female-biased dispersal with polyandry, and dispersal by both sexes with monogamy (Greenwood 1980). Because these processes are at the foundation of extinction-recolonisation dynamics, they play an important role in determining the vulnerability of species to ecological disturbance.

Disturbance is an intrinsic component of ecological communities worldwide, driving patterns of spatial and temporal heterogeneity (Sousa 1984, Turner 2010). Disturbance has been increasingly recognised as playing a crucial role in structuring and maintaining ecological communities by directly affecting mortality and reproduction, or by changing habitat suitability and landscape connectivity (Sousa 1984, Banks et al. 2013). Furthermore, because key biological processes such as reproduction and dispersal are influenced by patterns of habitat suitability, disturbance can constrain the abundance, distribution and movement of animals across the landscape (Templeton et al. 2011).

Fire

Fire is a major environmental disturbance that drives variation in habitat structure, resource availability and wildlife abundance in a number of different ecosystems (Russell-Smith et al. 1998, Lindenmayer et al. 2008, Bradstock et al. 2012). Fire events occur as part of a 'regime', which describes the historical series of these single events (Gill and Allan 2008). Typically, the fire regime takes into account fire intensity, frequency (between-fire interval), season of burn, fire type (above or below ground) and the extent of fires in the landscape (Gill 1975, Gill and Allan 2008). Fire has been a key process underlying many ecological communities since it appeared, about 420 million years ago (soon after the terrestrial plants) (Bowman et al. 2009). However, it is becoming clear that, through both direct and indirect human influence, historical patterns of wildfires

and fire regimes are beginning to change in a number of ecosystems around the world (Bowman et al. 2009, Turner 2010).

Australia is the most fire prone continent on Earth (Bradstock 2010). Although fires have always been a defining characteristic of the Australian landscape, research predicts that the size, frequency and severity of fires will increase in the future (Williams et al. 2001, Flannigan et al. 2009). Factors such as climate change, the spread of exotic grasses, changes in the distributions of native plants and human interactions (such as land use) have been predicted to influence fire regimes (Keane et al. 2004, Gill and Allan 2008, Bradstock 2010). However, these factors and their outcomes will vary across different ecosystems.

Changed fire activity has been observed in the grassland savannas of northern Australia. Since European settlement, there has been a shift from purposeful indigenous fire management to fire patterns that are dominated by extensive, high intensity wildfire that occurs late in the dry season (Russell-Smith et al. 2003, Legge et al. 2011). Indigenous people managed fire in northern Australian landscapes for millennia and traditional burning practices likely resulted in patchy, low-intensity fires that were mostly concentrated in the early to mid-dry season (Bowman 1998, Yibarbuk et al. 2001). However, during European settlement, indigenous people were forced from their land to missions and government settlements, meaning that they were no longer able to undertake land management activities (Bradstock et al. 2012). The resulting shift towards extensive late dry season wildfires presents a major risk to the biodiversity of northern Australia, as inappropriate fire regimes can cause major changes in community structure and increase the risk of extinction for many species (Driscoll et al. 2010, Woinarski et al. 2011).

Fire management

Robust strategies for managing fire with a strong ecological and empirical grounding are lacking for many ecosystems worldwide and are often based on public perception rather than a body of research (Williams et al. 2003, Bowman et al. 2009). This is because the effects of fire on biodiversity can be complex, as fires can burn differently within and

between vegetation types and this can have markedly different impacts on fauna (Lindenmayer et al. 2008). Furthermore, management practices usually focus on plant communities, as there is limited data on the survival and recovery process for different animal groups (Bradstock 2008, Driscoll et al. 2010). Nevertheless, ecologically sustainable fire management practices are needed if we are to aid biodiversity conservation.

Contemporary fire management supports the fire mosaic concept, where a fine-grained patchwork of fire ages promotes habitat heterogeneity (Driscoll et al. 2010, Kelly et al. 2012). In northern Australia, a variety of fire management programs exist, with the general aim of increasing early dry season burning to result in increased retention of long-unburnt vegetation and to reduce the incidence and extent of late dry season, unmanaged fire (Russell-Smith et al. 1997, 2003, Legge et al. 2011, Price et al. 2012, Murphy et al. 2015). This is carried out through prescribed burning, a process which involves the planned application of fire to a predetermined area, to achieve specific objectives. Evidence suggests that this type of burning practice, which was likely the customary fire management strategy under Aboriginal custodianship, leaves more long unburnt areas in the landscape than when left unmanaged (Bradstock et al. 2012, Skroblin et al. 2014).

The scale at which fire mosaics should be implemented, as well as the range of variability in fire frequency and intensity that would help conserve biodiversity, are still key questions that need to be addressed (Driscoll et al. 2010). For example, fire mosaics may not be effective if they are implemented at a scale that does not align with biological processes that support population recovery (like dispersal or habitat requirements). Furthermore, our current understanding of species' responses to fire is largely pattern based and lacks direct knowledge about the mechanisms underlying the post-fire recovery process (Driscoll et al. 2010, Banks et al. 2011). Therefore, studies elucidating the mechanisms behind population recovery after fire will help us gain a more thorough understanding of the consequences of fire on biodiversity and will allow us to implement fire management strategies that more effectively support conservation.

Northern Australia mammal declines

More mammal species have become extinct in Australia in the last 200 years than anywhere else in the world (Short and Smith 1994, Woinarski et al. 2011). Historically, these extinctions occurred inland and in temperate zones, with mammals in the northern tropics of Australia remaining relatively stable (Woinarski et al. 2011). However, in recent decades there has been a rapid decline in many small to medium sized mammal species throughout these northern savanna regions (Woinarski et al. 2001, 2011, Ziembicki et al. 2015). This is surprising, as northern Australia has often been considered a refuge for biodiversity, given the lack of apparent landscape modification. The cause of these declines is complex and likely synergistic (Ziembicki et al. 2015). However, research suggests that mammals can be sensitive even to single fire events and thus may be particularly vulnerable to regimes of extensive and frequent fires (Corbett et al. 2003, Legge et al. 2008). Therefore, the increasing intensity and frequency of extensive wildfires has been postulated as one of the major threats driving mammal declines (Woinarski et al. 2011, Lawes et al. 2015b, Ziembicki et al. 2015).

Of the major terrestrial mammal groups in Australia, native rodents represent the greatest number of extinct and threatened species (Braithwaite and Griffiths 1996, Lawes et al. 2015a). Research suggests that disturbance (fire and grazing by introduced herbivores such as cattle) can remove or degrade habitat used by native rodents for shelter, nesting and foraging, increasing the risk of predation (Kutt and Woinarski 2007, McGregor et al. 2015, 2016, Leahy et al. 2016). This supports the hypothesis that the interaction between fire, grazing and predation (particularly by the feral cat, *Felis catus*) is the primary cause of decline for both native rodents and small to medium sized marsupials (Ziembicki et al. 2015). In this way, fire can indirectly affect survival and reproductive output, which has implications for population recovery for these vulnerable species. For these reasons there is urgent need for studies increasing our understanding of the processes by which species respond to, and recover after fire, particularly for Australian native rodents.

Mechanisms for population recovery

The immediate demographic consequences that arise from fire shape subsequent population recovery. Characterising these initial demographic patterns as a 'starting point' for post-fire recovery is therefore critical for understanding the mechanisms by which population recovery proceeds (Banks et al. 2011). Patterns of animal survival or recolonisation are strongly influenced by dispersal ability, patterns of habitat utilisation and refugia (Bradstock 2008). Identifying the roles of these processes can guide management strategies, by determining whether post-fire recovery is driven by *in situ* survival of individuals in burnt areas, or by rapid, post-fire recolonisation from unburnt habitat (Banks et al. 2017).

Distinguishing whether recovery is primarily driven by *in situ* survival or recolonisation is fundamental to understanding the potential for ecosystems to recover after wildfire (Lindenmayer et al. 2005, Banks et al. 2011, 2017). This is because understanding these mechanisms can help us to identify the spatial components of fire regimes that are critical to maintaining mammals in the landscape. In order to characterise these patterns, knowledge of life history traits, dispersal capability and other demographic parameters are needed (Lindenmayer and Peakall 2000, Driscoll et al. 2010, Banks et al. 2011). Therefore, studies encompassing a range of approaches are essential to help improve fire management strategies and effectively preserve Australia's declining mammal fauna.

Using population genetics to make demographic inferences

Researchers often turn to genetic methods to gain insight into the ecological and behavioural processes occurring both within and among populations. This is partly due to the logistical difficulties of gaining direct demographic estimates in the field (Fontanillas et al. 2004; Lawson Handley & Perrin 2007). Furthermore, the combined use of genetics and field-based research has the potential to provide more information than can be gained using either in isolation. However, before using genetic data to explore behavioural, ecological and demographic questions, it is important to understand the scale at which these processes occur.

The levels at which genetic structure can be investigated has been a primary focus in population genetic theory since Wright's seminal work (Wright 1943; 1951). Demographic processes, such as mating and dispersal are likely to be detected over local scales, with patterns of genetic structure reflecting familial relationships and spatial clustering of related individuals (Storz 1999; Banks & Peakall 2012). At the population level, these processes still contribute to patterns of genetic structure across the landscape. However, since the population is the unit of analysis (rather than the individual), meta-population dynamics, genetic drift and historical connectivity become important at this scale (Gaggiotti et al. 2004). Thus, in order to gain a contemporary perspective on gene flow and investigate demographic questions, it is often necessary to look at patterns over a fine-scale (over a scale of meters rather than kilometres). This is especially important for small, inconspicuous species, where dispersal and social behaviours occur over this ultra-local scale (reviewed in Chapter 2, Appendix S1). For small mammals in particular, this perspective is essential for inferring mating systems, dispersal strategies and the response of populations to ecological disturbance.

Thesis outline

In this thesis, I use genetic analyses based on a range of different molecular marker types and genotyping techniques, to investigate demographic processes, fire response, and post-fire recovery in small mammal populations. By combining computer simulation modelling, a traditional field study, and different types of genetic data, my thesis aims to provide a greater understanding of the complex processes underlying small mammal populations than would be possible with any of these approaches alone. Below, I outline the different components of my PhD research, divided into four self-contained data chapters.

Chapter 2

Studies using genetic data to investigate dispersal and mating systems often focus on how these processes influence genetic structure across populations or social groups. However, our knowledge of how they shape spatial genetic patterns over a finer scale (tens – hundreds of metres) is limited. In Chapter 2, I investigate the effects of dispersal

and mating systems on fine-scale genetic structure using individual-level simulations based on the biology of an Australian native marsupial, the agile antechinus (*Antechinus agilis*). Through these simulations, I explore how comparing patterns between the sexes, across a range of markers with different inheritance modes (autosomal microsatellites, mitochondria and the Y chromosome) can help us to learn more about the demographic processes occurring in small mammal populations.

Chapter 3

The increasing availability of cost-effective, commercially available Next-Generation Sequencing (NGS) adds another dimension to research wishing to utilise genetic data. The task of choosing between molecular markers, bioinformatic pipelines and filtering strategies can be difficult for both experts and non-experts alike. In Chapter 3, I explore the outcomes of some of these choices on the biological conclusions drawn from genetic analyses, using the pale field-rat (*Rattus tunneyi*), a native Australian rodent from the Kimberley region of Western Australia, as a case study.

Chapter 4

The pale field-rat is one of many small mammal species currently declining across northern Australia. Evidence suggests that the interaction between altered fire regimes and other key threats is responsible for these declines. In Chapter 4, I present findings from a manipulative fire experiment and capture-mark-recapture study, in which I investigate habitat preferences, fire response and post-fire recovery in pale field-rat populations. I characterise the spatial distribution of surviving individuals immediately after fires of differing spatial scales and intensity. With this evidence, I make inferences about how spatial recovery processes might differ between lower intensity, 'patchy' fires (representative of early dry season, prescribed burns), compared to high intensity, 'thorough' fires (approximating late dry season, unmanaged fire).

Chapter 5

While a large body of research has concentrated on fire response in small mammals, studies rarely focus on post-fire population recovery. Genetic analyses have the potential to improve our understanding of the recovery process. This is because fire influences

biological processes like reproduction, mortality and dispersal, and so also impacts the underlying genetic variation within and among populations. However, to date, no study has yet combined demographic and genetic evidence to understand how small mammal populations recover after fire in northern Australia. In Chapter 5, I use a combination of genetic and demographic evidence to evaluate different recovery hypotheses (*in situ* survival versus recolonisation). I also determine whether recovery proceeds differently depending on the spatial extent and patchiness of fires.

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Chapter 2

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The impact of mating systems and dispersal on fine-scale genetic structure at maternally, paternally and biparentally inherited markers

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Abstract

For decades, studies have focused on how dispersal and mating systems influence genetic structure across populations or social groups. However, we still lack a thorough understanding of how these processes and their interaction, shape spatial genetic patterns over a finer scale (tens – hundreds of metres). Using uniparentally inherited markers may help answer these questions, yet their potential has not been fully explored. Here, we use individual-level simulations to investigate the effects of dispersal and mating system on fine-scale genetic structure at autosomal, mitochondrial and Y chromosome markers. Using genetic spatial autocorrelation analysis, we found that dispersal was the major driver of fine-scale genetic structure across maternally, paternally and biparentally inherited markers. However, when dispersal was restricted (mean distance = 100 m), variation in mating behaviour created strong differences in the comparative level of structure detected at maternally and paternally inherited markers. Promiscuity reduced spatial genetic structure at Y chromosome loci (relative to monogamy), whereas structure increased under polygyny. In contrast, mitochondrial and autosomal markers were robust to differences in the specific mating system, although genetic structure increased across all markers when reproductive success was skewed towards fewer individuals. Comparing males and females at Y chromosome *versus* mitochondrial markers respectively, revealed that some mating systems can generate similar patterns to those expected under sex-biased dispersal. This demonstrates the need for caution when inferring ecological and behavioural processes from genetic results. Comparing patterns between the sexes, across a range of marker types may help us tease apart the processes shaping fine-scale genetic structure.

Introduction

A wide range of biological processes can influence patterns of genetic structure within and among populations. This has inspired the extensive use of genetic analyses to understand behavioural and ecological patterns (Chesser 1991a; Mossman & Waser 1999; Ross 2001; Banks & Peakall 2012; Parreira & Chikhi 2015). Of particular interest has been the use of genetic analyses to identify patterns of animal movement (Goudet *et al.* 2002; Lawson Handley & Perrin 2007; Banks & Peakall 2012). However, genetic structure can be influenced by a many behavioural, ecological and molecular processes other than dispersal, such as social structure and mating systems (Sugg *et al.* 1996; Storz 1999; Parreira & Chikhi 2015). Furthermore, these processes can influence genetic structure differently across markers with different inheritance modes (Chesser & Baker 1996; Petit *et al.* 2002; Hedrick 2007; Greminger *et al.* 2010). Thus, understanding the impact these factors have on genetic patterns may help us avoid false conclusions about ecological and behavioural processes.

Comparing patterns across different marker types presents an exciting opportunity for biological inference from genetic data. Until recently, studies using this comparative marker approach in species other than primates, focused mainly on comparing autosomal markers with the maternally inherited mitochondria (mtDNA) (Sunnucks 2000; Petit *et al.* 2002; Prugnolle & de Meeus 2002; Hedrick *et al.* 2013). However, in mammals mtDNA markers make an ideal comparison to the Y chromosome, as both are inherited from one parent and are non-recombining, or have non-recombining regions, which are preserved as haplotypes during sexual reproduction (Petit *et al.* 2002; Prugnolle & de Meeus 2002; Greminger *et al.* 2010). Alternatively, while the X chromosome spends less evolutionary time in the male germ line compared to autosomal markers, it is not uniparentally inherited. This means that the X and Y chromosomes are not directly comparable (MacDonald *et al.* 2014). However, comparing Y chromosome to mtDNA markers may provide a sex-specific genetic perspective for inferring biological processes (Goudet *et al.* 2002; Petit *et al.* 2002; Lawson Handley & Perrin 2007). Furthermore, these markers may offer insight into these processes over greater time scales, as both

uniparental inheritance and the lack of recombination ensure genetic patterns are maintained.

Development of Y chromosome markers in wild populations remains rare, partly due to low levels of polymorphism at the Y chromosome (Petit *et al.* 2002; Greminger *et al.* 2010; Evans *et al.* 2014). However, studies using the Y chromosome are becoming more feasible with next generation sequencing and reference genome information (Petit *et al.* 2002; Greminger *et al.* 2010; Neaves *et al.* 2013; MacDonald *et al.* 2014). In fact, a growing number of studies are using population-level analyses of the Y chromosome in combination with other genome regions to find evidence for sex-biased dispersal (Hammond *et al.* 2006; Schubert *et al.* 2011; Yannic *et al.* 2012; MacDonald *et al.* 2014), skewed sex ratios and polygyny (Neaves *et al.* 2013), population expansion and contraction, and variation in mutation rates between the sexes (Evans *et al.* 2014).

In order to take full potential of uniparentally inherited markers in population genetic studies, it is fundamental that we understand how these markers are influenced by ecological and behavioural processes. A number of simulation studies have investigated the ability of autosomal markers to detect differences in genetic structure between the sexes, both at an individual and population level (Goudet *et al.* 2002; Banks & Peakall 2012; Parreira & Chikhi 2015). However, the potential to use uniparentally inherited markers at the individual level, rather than at population or social group levels, has not been extensively explored. This is a major knowledge gap, as the effect of social behaviours and dispersal are likely to be particularly important for influencing the distribution of individual genotypes and haplotypes in space (Banks & Peakall 2012; van Dijk *et al.* 2015).

Genetic data provide powerful tools for elucidating processes such as dispersal and mating behaviour, but any inferences made from such data should be strongly grounded in an understanding of the genetic patterns expected under the diverse mating and dispersal strategies that occur (McEachern *et al.* 2009; Blyton *et al.* 2012; also, see Appendix S1, for an extensive list of mammalian examples). When considering these processes in mammals, there is a long held assumption that most species are polygynous

and dispersal is male biased (Greenwood 1980; Foltz 1981). However, this assumption tends to overlook small and inconspicuous species, where dispersal and social behaviours occur over much finer scales (Foltz 1981; Burda *et al.* 2000; Swilling & Wooten 2002; Maher & Duron 2010). These processes can vary across species (e.g. bats show a range of complex social, mating and dispersal patterns, see Kerth 2008), as well as within single populations (depending on temporal, spatial, demographic or environmental variables, see: Busch *et al.* 2009; Yannic *et al.* 2012; Keane *et al.* 2015). It is not surprising then, that patterns detected in genetic investigations often do not reflect the mating systems or dispersal patterns previously identified in observational studies (McEachern *et al.* 2009). Thus, to accurately interpret genetic data, it is essential to understand how mating systems and dispersal influence patterns of genetic structure.

Here, we use spatially explicit, individual-level simulations to investigate a range of dispersal and mating scenarios found across small mammal species (Fig. 1) and their effect on fine-scale spatial genetic structure as measured by spatial autocorrelation (Smouse & Peakall 1999; Peakall *et al.* 2003; Smouse *et al.* 2008; Banks & Peakall 2012; Blyton *et al.* 2015). We define fine-scale genetic structure as the non-random distribution of genotypes and haplotypes in space, over spatial scales of tens to hundreds of metres (Banks & Peakall 2012). Simulations provide a powerful and flexible tool for exploring different biological processes, and can be adapted to investigate many different ecological and behavioural scenarios.

As a starting point, simulations were built around the life history of the agile antechinus (*Antechinus agilis*), an Australian marsupial with a long history as a study organism in behavioural, landscape and molecular ecology (Cockburn *et al.* 1985; Kraaijeveld-Smit *et al.* 2002a; b; c; Banks *et al.* 2005a; Fisher *et al.* 2006a; b; Banks & Lindenmayer 2013). Simulations were then extended to test hypotheses relating to a range of dispersal and mating system scenarios observed across small mammal species (ensuring relevance to a wide range of real world scenarios). Simulations are therefore broadly representative of mammalian systems where females produce multiple offspring in a single litter, for a range of common mating and dispersal strategies. We compare the level of fine-scale genetic structure between females and males to provide insights into

the ecological questions that can be answered using the combination of Y chromosome, mtDNA and autosomal markers.

We explore three key hypotheses related to both mating and dispersal: (1) fine-scale genetic structure across autosomal, mtDNA and Y chromosome markers will be strongly influenced by dispersal, with limited dispersal increasing fine-scale genetic structure and high levels of dispersal reducing this structure. (2) When comparing Y chromosome with mtDNA markers (paternally and maternally inherited markers), varying the mating system from promiscuity to monogamy and polygyny will influence fine-scale genetic structure differently for females and males. (3) Increased reproductive success under promiscuity (females) and polygyny (males) will lead to increased fine-scale genetic structure at autosomal, mtDNA and Y chromosome markers.

Methods

Several life history traits of the agile antechinus provide rich opportunities for simulation-based testing (Banks & Peakall 2012). This semelparous dasyurid marsupial is commonly found in south-eastern Australia. Promiscuous mating occurs in the same week each year and individuals mate in their first breeding season after birth. All males die after this breeding season and very few females survive to reach a second breeding year, resulting in almost completely discrete generations (Cockburn *et al.* 1985; Naylor *et al.* 2008). Females can have up to 10 young, with most litters sired by two or three males; however, as many as seven sires for a single litter have been found (Kraaijeveld-Smit *et al.* 2002b; Banks *et al.* 2005a). After weaning, almost all juvenile males disperse, whereas females remain strongly philopatric (male-biased dispersal; Cockburn *et al.* 1985; Banks *et al.* 2005a). Daily movements for most individuals are less than 100 m, although social home ranges vary between the sexes (Lazenby-Cohen & Cockburn 1991; Banks & Peakall 2012). Over a multi-year study, the social range for females never exceeded 3 ha on average, whereas males could exceed 5 ha on average (Lazenby-Cohen & Cockburn 1991).

Simulation details

Spatially explicit genetic simulations were conducted using an extended version of the software package GenAlEx 6.5 (Peakall & Smouse 2006, 2012). The simulation process is well documented in previous studies by Banks & Peakall (2012) and Blyton *et al.* (2015), and simulations are extensively validated in the supplementary data for these papers. Here, we added the capability to output haplotypes for mtDNA and Y chromosome markers and to vary reproductive parameters.

After defining parameters, we simulated mating and dispersal to create spatially referenced, autosomal genotypes and mtDNA and Y chromosome haplotypes for all individuals within the simulation landscape. Simulations were performed over a continuous, hypothetical 5.6 x 5.6 kilometre landscape, with a total carrying capacity of 15700 individuals and an equal sex ratio. Density was controlled following Banks & Peakall (2012) and Blyton *et al.* (2015), with a mean of 5 and maximum of 10 individuals ha^{-1}, consistent with findings for density in real populations (Banks *et al.* 2005a). At the end of each simulation, we subsampled 500 individuals for analysis from the central 100 ha, as previous work revealed that differences in spatial autocorrelation patterns between the sexes are most readily detected at or below the scale over which dispersal is limited in the philopatric sex (Banks & Peakall 2012). This is also true for behavioural processes, which are likely to occur over the scale of a home range (Banks & Peakall 2012; Blyton *et al.* 2015). A focused sampling effort (rather than sampling spread over many kilometres) is therefore most likely to detect meaningful differences in spatial autocorrelation patterns between the sexes (Banks & Peakall 2012). Furthermore, the scaling of dispersal, population density and sampling in our simulations is likely to be indicative of many empirical studies of small mammals and represents a feasible sampling design. The relative scaling of these processes should also be applicable to many molecular ecological studies of similar processes in other taxa.

Overview of the simulation process

Simulations began with the setup of initial allele and haplotype frequency distributions, drawn at random from an even distribution of 10 autosomal loci with 10 alleles each and 10 mtDNA and Y chromosome haplotypes. In reality, the number of unique mtDNA and Y

chromosome haplotypes identified varies considerably among studies and taxa. However, we chose to use 10 haplotypes as this is representative of real situations, with many population level studies finding between 1–18 mtDNA and Y chromosome haplotypes within populations, at the sequences analysed (e.g. in birds: Johnson *et al.* 2003; Pierson *et al.* 2010, mammals: Eriksson *et al.* 2006; Nietlisbach *et al.* 2012, and reptiles: Ujvari *et al.* 2008). Furthermore, exploratory analyses revealed that variation in the number of loci, alleles and haplotypes did not dramatically alter patterns of genetic structure, but did influence the power of spatial autocorrelation analysis (Appendices S2 – S3). This is particularly important for directly comparing mtDNA and Y chromosome markers, since the number of haplotypes generally differs between markers in empirical data.

Genotypes and haplotypes were randomly constructed from pre-defined allele and haplotype frequency distributions and sex and XY coordinates were randomly allocated. The first generation was obtained by random mating among all individuals in the population (establishing Hardy-Weinberg equilibrium), with offspring becoming parents in the following generation. After this initial random generation, mating included nearest neighbours only. Sires were drawn from a list of potential nearest neighbour mates (calculated from pairwise geographical distances among individuals), with a mean of 72–76 m, approximating the distance over which females select male antechinus in the wild (0–200 m; Banks *et al.* 2005b). When simulating polygyny, this distance was reduced to an average of ~30 m, owing to the parameter set changes required to represent the harem structure usually associated with this mating system (for detailed information on mate search distances across all mating systems, see Appendix S4). Inbreeding avoidance mechanisms were not included in simulation parameters (with the exception of sex-biased dispersal, detailed below). These mechanisms are unlikely to be important for our results given that we measured fine-scale genetic structure within same sex individuals (and only then compared between the sexes). However, this could be investigated by comparing opposite-sex pairs (see Blyton *et al.* 2015). Following mating, female and male offspring were dispersed.

In a genetic mark-recapture study, Banks (2005) found that juvenile males dispersed 1250 m on average (median 274 m; maximum 6000 m). However, males of the closely related *Antechinus stuartii* only dispersed a mean distance of 387 m (median 303 m; maximum 1230 m; Fisher 2005; Banks *et al.* 2011). In both studies, female mean dispersal was <100 m. Therefore, in our simulations dispersal distances were drawn from an exponential distribution with a mean dispersal distance of 100 m representing philopatry or restricted dispersal, and a mean dispersal distance of 500 m representing high dispersal (2.5–97.5 percentiles of dispersal distances: restricted dispersal = 2.6 m – 407.5 m; high dispersal = 12.8 m – 1864 m. For distributions of dispersal distances, see Appendix S5). The direction in which an individual dispersed was decided by drawing a random angle from 0° to 360°. If the resulting coordinates were already at maximum density, this process (allocating dispersal distance and direction) was repeated until an available location was found, for a maximum of 20 search loops.

We ran all simulations for 100 generations, as exploratory analyses indicated that fine-scale genetic structure develops quickly, but can take 10–15 generations to fully stabilise (Appendix S6 and Banks & Peakall 2012). Female and male genetic (autosomal, mtDNA and Y chromosome) and geographical distance matrices were output at the 100th generation, after dispersal had occurred. This process was repeated for 100 simulations, with a new population created at the beginning of each simulation.

Simulation parameters

Simulation parameters were divided into two categories, those that were fixed throughout this study (and drawn from the biology of the agile antechinus) and those that were varied. Fixed parameters included non-overlapping generations that lasted one year, an equal sex ratio and a mean population density of five animals per hectare, with a maximum density of 10. The maximum number of offspring for both sexes was held at 10 for all simulations (Banks *et al.* 2005b). Several other parameters were varied in order to ask the following questions:

What is the effect of dispersal on fine-scale genetic structure at autosomal, mtDNA and Y chromosome markers?

We simulated three different dispersal scenarios by changing the mean exponential dispersal distance for females and males. Male-biased dispersal (consistent with the antechinus system) was modelled by setting mean dispersal distance to 100 metres for females and 500 metres for males (hereafter simplified as F100/M500). Restricted dispersal (or philopatry) was modelled by setting both male and female mean dispersal distance to 100 metres (F100/M100). This dispersal scenario was also simulated to represent sampling individuals pre dispersal (as individuals within the same litter and neighbouring litters remained spatially clustered when the mean dispersal distance was 100 m). Finally, high dispersal was modelled by setting the mean dispersal distances for both sexes to 500 metres (F500/M500). We did not investigate less extreme levels of sex-biased dispersal as previous research using autosomal markers suggests that when one sex is strongly philopatric, the signals of sex-biased dispersal develop rapidly, even when this bias is subtle (Banks & Peakall 2012).

What is the effect of the mating system on fine-scale genetic structure at autosomal, mtDNA and Y chromosome markers?

We simulated three common mating strategies by varying a range of parameters under each of the above dispersal scenarios (see Fig. 2 for a detailed infographic describing this process, with predictions for how these processes influence fine-scale spatial genetic structure at mtDNA and Y chromosome markers). We simulated promiscuity (consistent with the antechinus system), monogamy and polygyny. In all three cases, females could produce an average of three offspring ($\lambda = 3$) with the allocation of offspring to females following a Poisson distribution with the maximum number of offspring capped at 10. In each generation, females were randomly selected for mating until the carrying capacity was reached. The number of females contributing to reproduction and the average number of offspring produced by each female did not differ substantially between promiscuity ($\lambda = 3$), monogamy and polygyny. Conversely, the number of males contributing to reproduction and the average number of offspring produced by each male differed dramatically between mating systems (see below, as well as Appendices S7 – S9, for detailed parent and offspring data).

Promiscuity was modelled by allowing a maximum of five males to contribute to the paternity of a litter with the mean number of sires per litter approximately 2.75. Sires were drawn from the 10 nearest neighbours. On average (over all 100 simulations), 4978 females contributed to reproduction compared to 6014 males, from a total of 15700 individuals. Females produced a mean of 3.15 offspring, whereas males produced a mean of 2.61.

Monogamy was modelled by reducing the number of sires per litter to one and specifying that males were only able to mate once. An average of 4934 individuals of each sex contributed to reproduction and both females and males produced 3.02 offspring on average. This meant that the number of males contributing to reproduction decreased by 18% and the mean number of offspring per male increased by 16% relative to promiscuity ($\lambda = 3$).

To represent polygyny, the maximum number of sires per litter and the number of nearest neighbours were reduced to one, effectively forcing females to mate with only one male. However, males could be the nearest neighbour for multiple females, meaning they were able to mate more than once. Therefore, a smaller number of males were producing more offspring, across multiple litters. The mean number of offspring produced by males increased by 74% to 4.55 and the number of males contributing to reproduction decreased by approximately 43% to 3451, relative to promiscuity ($\lambda = 3$) (females = 3.16 and 4975 respectively). Under polygyny, it was possible for one male to sire only one litter, thus monogamy could also occur. However, this is also a possibility in real populations and would weaken any sex-specific differences in fine-scale spatial genetic structure caused by the mating system, meaning that conclusions were drawn from conservative estimates of sex-specific differences in structure.

What is the effect of reproductive skew on fine-scale genetic structure at autosomal, mtDNA and Y chromosome markers?

In many real world cases, only a subset of individuals successfully reproduce, such that mating success is strongly skewed. To explore this component of reproductive biology,

we investigated the impact of increasing levels of reproductive skew for both females and males across all dispersal scenarios. Extreme female reproductive skew was investigated under promiscuity by changing the mean number of offspring produced by females (λ) from 3 to 8, meaning females produced larger litters. By increasing the litter size, the carrying capacity of the population was reached before the majority of females reproduced, thus skewing reproduction in favour of a small number of females. This resulted in a 58% decrease in the number of females contributing to reproduction (mean = 2070) and the mean number of offspring produced by each female increased by 141% (mean = 7.58) (compared to promiscuity, λ = 3). Male reproductive skew also increased, but only slightly, with the number of males contributing to reproduction decreasing by17% (mean = 5008) and the number of offspring produced by each male increasing by 20% (mean = 3.13; compared to promiscuity, λ = 3).

Moderate male reproductive skew was investigated under polygyny, as in this mating system reproductive success is skewed towards fewer males (43% fewer males than under promiscuity (λ = 3), mean = 3451). Under polygyny, males produced more offspring than under any other mating system (mean = 4.55).

Statistical analysis

We compared simulation results between females and males at autosomal, mtDNA and Y chromosome markers. Simulations were analysed in GenAlEx 6.5 (Peakall & Smouse 2006, 2012) using the genetic distance based method of multilocus spatial autocorrelation analysis. This method allows any data type to be used (e.g. multilocus allelic genotypes, biallelic SNPs or haplotypes) and measures the relationship between genetic and geographical distance by estimating the autocorrelation coefficient, *r*, for each group of individuals over specified distance classes (Smouse & Peakall 1999; Peakall *et al.* 2003; Double *et al.* 2005; Smouse *et al.* 2008). This coefficient is bounded by [-1 +1] and is related to Moran's *I*, with high *r* values representing high levels of relatedness over a particular area. Following Banks & Peakall (2012), *r* was estimated for five distance classes of 100 metres each (500 metres in total), as this optimised both the scale of fine-scale genetic structure and the sample size needed for detecting this structure. We used known home range size and dispersal distances to inform our choice for these distance

classes, however in species where this data in unavailable, exploratory analyses can be used to determine the most biologically relevant distance classes (as outlined in Peakall *et al.* 2003; Beck *et al.* 2008).

We compared the distribution of male and female *r* values over 100 simulations at all three markers to investigate whether different behavioural and ecological processes drive sex-specific differences in fine-scale spatial genetic structure. The null hypothesis predicts no difference in fine-scale genetic structure between the sexes ($r_{females} = r_{males}$). However, if the alternative hypothesis is true, then one sex will show higher levels of fine-scale genetic structure than the other. To investigate this, we looked at the distribution of differences in female and male *r* values ($r_{females} - r_{males}$) in the first distance class, because genetic structure is more apparent at this finer scale (Banks & Peakall 2012). Under no difference in fine-scale genetic structure between the sexes, this distribution is centred on zero. However, differences in fine-scale genetic structure between the sexes will shift the distribution in a positive or negative direction (positive = $r_{females} > r_{males}$, negative = $r_{females} < r_{males}$).

To test whether differences in spatial autocorrelation patterns between the sexes were significant, we compared 95% bootstrap confidence intervals (CIs) about the autocorrelation *r* values within each individual simulation, following Peakall *et al.* (2003). Banks & Peakall (2012) showed by simulation that this approach is consistent and conservative for both type I (falsely rejecting the null hypothesis) and type II errors (falsely rejecting the alternative hypothesis). Bootstrap 95% CIs were estimated for *r* by drawing (with replacement) from a set of pairwise comparisons in the first distance class (Smouse & Peakall 1999). We then tallied the number of simulations in which female and male Bootstrap 95% CIs did not overlap (indicating a significant difference in fine-scale spatial genetic structure between the sexes).

Results

Simulation performance was extensively validated and returned the results expected relative to the parameters set (see Appendices S2 – S9). Spatial autocorrelation *r* values

were strongly influenced by varying the mean dispersal distance for females and males (Fig. 3). This was most apparent at the first distance class (0–100 metres), with genetic spatial autocorrelation *r* values decreasing to zero by the fifth distance class (400–500 metres). This was true for all markers and for all dispersal scenarios. Below, our results focus on the magnitude of *r* values in the first 100 m distance class, as this provides the most informative metric for investigating the effects of the biological processes modelled.

Male-biased dispersal (F100/M500)

Promiscuity (λ= 3)

When simulation parameters were realistic to the antechinus system, autocorrelation *r* values were substantially higher in females than males across all three markers [Mean *r* for autosomal = F: 0.033 vs. M: 0.004; mtDNA = F: 0.15 vs. M: 0.026; mtDNA vs. Y chromosome = F: 0.15 vs. M: 0.005 (Table 1; Fig. 4: column b)]. Across all simulations, $r_{females} - r_{males}$ (the distribution of the difference between female and male *r*) was positive and did not overlap zero, meaning that female *r* was always greater than male *r* (Fig. 4: column b.). Across the different marker types, female and male 95% bootstrap CIs did not overlap in 92–99 of 100 simulations (Appendix S10). The correlograms for all markers showed this typical pattern of male-biased dispersal, with non-overlapping 2.5–97.5 percentiles for the distributions of *r* values for females and males (Fig. 3: column b).

Monogamy, polygyny, promiscuity (λ= 8)

Varying the mating system from promiscuity (λ= 3) to monogamy and polygyny had no apparent influence on patterns of genetic spatial autocorrelation when dispersal was male-biased (Table 1). Females showed higher levels of fine-scale spatial genetic structure than males across all marker types (Fig. 4: column b). Furthermore, female and male 95% bootstrap CIs did not overlap in 95–100 simulations (Appendix S10).

High male dispersal removed any impact of increased male reproductive skew under polygyny (Fig. 4: column b). However, in females (where dispersal was restricted), increasing female reproductive skew under promiscuity (λ= 8) resulted in higher levels of

fine-scale genetic structure at autosomal and mtDNA markers [mean *r* for promiscuity λ= 3 vs. promiscuity λ= 8: autosomal = 0.033 vs. 0.058; mtDNA= 0.150 vs. 0.283 (Table 1; Fig. 4: column b)]. $r_{females}$ - r_{males} was therefore greater than under any other mating system (Fig. 4: column b) (with the exception of the difference found under polygyny at autosomal markers, which was similar to promiscuity λ= 8). Non-overlapping 95% bootstrap CIs were seen in 99–100 simulations (Appendix S10).

Restricted dispersal for both sexes (F100/M100)

Reducing mean dispersal distance to 100 metres created strong patterns of spatial autocorrelation for both females and males, with positive distributions of simulated *r* values across all mating scenarios at autosomal, mtDNA and Y chromosome markers (Fig. 4: column a; Table 1). However, despite equal, restricted dispersal for both sexes, variation in mating system generated different patterns of genetic spatial autocorrelation between females and males when comparing Y chromosome with mtDNA markers (Fig. 4: column a).

Y chromosome versus mtDNA markers

Promiscuity (λ= 3)

Under promiscuity (λ= 3), female mtDNA *r* values were greater than male Y chromosome *r* values [mean *r* for mtDNA = F: 0.137; Y chromosome = M: 0.087 (Table 1)]. $r_{females}$ - r_{males} overlapped zero, but was skewed towards positive values, meaning that in most cases female fine-scale spatial genetic structure was greater than that of males (Fig. 4: column a). Female and male 95% bootstrap CIs did not overlap in 36 simulations (Appendix S10).

Polygyny

Under polygyny, the reverse pattern was found, with males having considerably higher autocorrelation *r* values than females [mean *r* for mtDNA = F: 0.148; Y chromosome = M: 0.214 (Table 1)]. While $r_{females}$ - r_{males} overlapped zero, the distribution was strongly skewed towards negative values, indicating that male fine-scale spatial genetic structure was greater than that of females in the majority of simulations (Fig. 4: column a). Of the

100 simulations, 51 showed non-overlapping 95% bootstrap CIs between the sexes (Appendix S10).

Monogamy

Monogamy resulted in similar distributions of simulated *r* values between females and males [mean *r* for mtDNA = F: 0.111; Y chromosome = M: 0.096 (Table 1)], with $r_{females}$ - r_{males} bounding zero (Fig. 4: column a). In 14 simulations, female and male 95% bootstrap CIs did not overlap (Appendix S10). Given the equal dispersal and mating opportunities present under monogamy, we would expect no difference in fine-scale genetic structure between the sexes. However, this skew towards increased female structure is driven by the dispersal component of the mating system (mate-search dispersal, see Appendix S4). However, the difference in female and male fine-scale genetic structure driven by mate-search dispersal is much less pronounced than the differences driven by the actual mating behaviours (which individuals mate) across each mating system.

Promiscuity (λ= 8)

Increased female reproductive skew under promiscuity resulted in substantially higher autocorrelation *r* values for females than males [mean *r* for mtDNA = F: 0.255; Y chromosome = M: 0.111 (Table 1)], generating a similar pattern to that seen under male-biased dispersal (Fig. 4: column a). This resulted in a substantial divergence between female and male distributions of simulated *r* values, with $r_{females}$ - r_{males} strongly positive and not overlapping zero (Fig. 4: column a). Female and male 95% bootstrap CIs did not overlap in 84 simulations (Appendix S10), with these results approaching those found under male-biased dispersal (where 92–100 simulations showed non-overlapping 95% bootstrap CIs between the sexes).

Autosomal and mtDNA markers

All mating systems

When comparing females and males at autosomal and mtDNA markers, variation in mating system influenced the magnitude of simulated *r* values, but patterns of fine-scale spatial genetic structure were consistent between the sexes. Under each of the four

mating scenarios, female and male distributions of simulated *r* values mirrored each other, with $r_{females}$ - r_{males} bounding zero (Fig. 4: column a; Table 1). Only a small number of these simulations (3–9) showed non-overlapping 95% bootstrap CIs between the sexes (Appendix S10). At mtDNA markers, increased female reproductive skew under promiscuity (λ= 8) created higher levels of fine-scale spatial genetic structure for both sexes. At autosomal markers, male and female fine-scale spatial genetic structure increased under both promiscuity (λ = 8, increased female reproductive skew) and polygyny (increased male reproductive skew) (Fig. 4: column a; Table 1).

High dispersal for both sexes (F500/M500)

All mating systems

When high levels of dispersal were present for both sexes, variation in mating system had no obvious impact on fine-scale spatial genetic structure (Table 1; Fig. 4: column c). Genetic spatial autocorrelation was not present for males or females across all markers and all mating systems. There was no apparent difference between the distributions of female and male simulated *r* values and $r_{females}$ - r_{males} was centred on zero (Fig. 4: column c). Only 0–2 simulations showed non-overlapping 95% bootstrap CIs between the sexes, across all markers and mating scenarios (Appendix S10).

Discussion

The impacts of social and behavioural processes on genetic structure are often overlooked in studies focused on dispersal. Here, we have developed a simulation framework to help us understand the processes that contribute to patterns of fine-scale spatial genetic structure across uniparentally and biparentally inherited markers. We found that dispersal was the major driver of fine-scale spatial genetic structure, with limited dispersal distances generating strong patterns of fine-scale genetic structure and high dispersal removing this structure. Sex-biased dispersal is expected to generate a significant difference in fine-scale genetic structure between the sexes (Banks & Peakall 2012). Indeed, in this study, we found that under male-biased dispersal, females consistently showed greater genetic structure than males across all marker types and

mating systems. Furthermore, female and male 95% bootstrap CIs did not overlap in 92–100% of simulations. This means, when considering a single point analysis (such as one would carry out in an empirical study), there was a 92–100% chance that a significant difference in fine-scale genetic structure would be detected between the sexes.

Along with this compelling evidence that dispersal is a major driver of fine-scale spatial genetic structure, our comparison of male Y chromosome with female mtDNA markers revealed that mating systems can also strongly influence patterns of fine-scale spatial genetic structure under restricted dispersal. Critically, promiscuity (λ= 3 and 8) and polygyny, while opposite, created a result similar to that expected under sex-biased dispersal in the absence of any dispersal bias. For example, when considering a single point analysis there was a 36–84% chance of detecting a significant difference between female and male fine-scale genetic structure, generated by mating system alone. In contrast, mtDNA and autosomal markers were fairly robust across different mating systems, but fine-scale spatial genetic structure increased at both marker types when reproductive success was skewed towards fewer individuals. These findings have important implications for any studies intending to infer ecological and behavioural processes from genetic data, which we discuss in detail below.

Mating systems and reproductive skew

When simulated dispersal distance was low for both sexes, the level of fine-scale genetic structure differed between Y chromosome markers in males and mtDNA markers in females depending on the mating system, despite identical dispersal patterns for both sexes. Under promiscuity, higher levels of positive genetic spatial autocorrelation were present in females than in males. Under polygyny, this was reversed, with male genetic spatial autocorrelation almost always greater than that of females. The comparative difference in the level of fine-scale genetic structure between the sexes was driven by male Y chromosome markers (see Figure 2).

An explanation of these patterns is offered by considering the consequences of each mating system on Y chromosome diversity. Promiscuity (and likely polyandry, though not simulated here) reduces the probability that Y chromosomes are identical by

descent within litters, while polygyny increases the probability of identical by descent Y chromosomes among litters. This increases local Y chromosome diversity within litters or reduces local Y chromosome diversity among litters, thereby shaping fine-scale spatial genetic structure in the relevant groups. These results highlight the influence of mating systems and sociality in driving patterns of genetic diversity, particularly at uniparentally inherited markers. Indeed, Parreira & Chikhi (2015) used simulations and comparisons with real data from ecological and population genetic studies to show that sociality can maintain genetic diversity without the need for sex-biased dispersal or other inbreeding avoidance mechanisms. This suggests that social behaviours, such as mating strategies, are an important aspect of genetic structure and need to be accounted for in genetic studies. It is important to note, however, that mating systems can also facilitate gene flow through additional movement in the form of mate searching. The distance over which individuals choose mates can vary considerably among species and can impact patterns of gene flow across the landscape (Double *et al.* 2005). Using simulations, Blyton *et al.* (2015) showed that as the spatial scale over which individuals chose mates increased, spatial genetic structure decreased. Indeed, in our study, we found that mate-searching movements by males slightly reduced fine-scale genetic structure (as seen under monogamy). However, mating behaviour (which individuals were involved in mating) still had a much more pronounced impact on fine-scale genetic structure than this dispersal component of the mating system.

Increasing reproductive skew for females under promiscuity generated substantially higher levels of fine-scale spatial genetic structure at mtDNA markers in our simulations. This is likely because the population consisted of a relatively smaller number of larger litters with identical maternally inherited mtDNA. Similarly, polygyny increased fine-scale spatial genetic structure for males at Y chromosome markers, due to fewer males producing more offspring and siring entire litters with identical paternally inherited Y chromosomes (rather than producing fewer offspring across litters with multiple sires). Eldon & Wakeley (2006) used simulations and an empirical study of Pacific oysters to show that reproductive skew is an important factor for describing levels of genetic diversity across populations. Our results demonstrate that reproductive skew can also be important over finer-scales, as the effects on genetic variation described above will be

exaggerated by litter size and will vary depending on the mating system. For example, increased male reproductive skew under promiscuity may counteract the reduction in genetic structure caused by multiple mating, thus resulting in similar levels of fine-scale structure for both sexes. Therefore, while the mating system creates differences in female and male genetic structure, the level of reproductive skew determines how extreme this difference will be.

In species where females only produce one or two offspring every year (or every few years) and the majority of females successfully reproduce, such as in mountain brushtail possums (Lindenmayer *et al.* 1998; Blyton *et al.* 2015) or white-tailed deer (Verme 1965), fine-scale genetic structure at maternally inherited markers would be expected to be low compared to species with large litters (all else, including dispersal, being equal). Conversely, in species where females produce thousands of offspring at a time, such as marine invertebrates (Hedgecock 1994), or in systems where a small number of females dominate reproduction, such as naked mole rats (Clarke & Faulkes 1997; Patzenhauerová *et al.* 2013), genetic structure at maternally inherited markers would be expected to be very high (in the absence of differences in dispersal). At Y chromosome markers, promiscuity, polyandry, polygyny and the number of males contributing to reproduction are all important factors for shaping fine-scale spatial genetic structure. However, these factors may also have a greater impact when females can produce more offspring.

Dispersal

Dispersal had the largest impact on the magnitude and direction of fine-scale genetic structure and generally outweighed any influence of the mating system. High dispersal created low or no positive genetic spatial autocorrelation across all marker types and removed the effect of mating system on genetic structure differences between Y chromosome and mtDNA markers. When male dispersal was high, but females remained mostly philopatric, females always showed higher levels of positive genetic spatial autocorrelation than males (significant in 95-100% of simulations). Thus, philopatry plays

an important role in allowing the detection of genetic structure developed under sociality.

Previous studies have demonstrated that social dynamics can have a major influence on the magnitude of population genetic structure, so long as some degree of philopatry is present (Chesser 1991b; Dobson *et al.* 1997, 1998; Storz 1999). For example, in greater spear-nosed bats, one successful male may sire over 50 offspring in his reproductive lifetime, whereas the majority of males will never successfully reproduce (McCracken & Bradbury 1981). Despite this extreme skew in mating success, greater spear-nosed bats showed a relatively low level of population differentiation (F_{ST} = 0.031), most likely driven by the fact that juveniles of both sexes disperse in this species (McCracken & Bradbury 1977, 1981; McCracken 1987). Conversely, red howler monkeys also exhibit a polygynous mating system, where females live in harems and a single male usually sires the majority of offspring (Pope 1990). However, in this species among-group differentiation was high (F_{ST} = 0.142–0.225), likely driven by the fact that ~33% of female red howler monkeys remain philopatric (Pope 1992). Therefore, high dispersal in greater spear-nosed bats randomly distributed genetic variation across the total population, removing any patterns of population-level genetic structure generated by the mating system. In contrast, female philopatry in red howler monkeys reinforced the population-level genetic structure developed under polygyny, creating genetically differentiated groups (Storz 1999).

The interplay between dispersal and mating strategies has long been known to influence patterns of genetic variation (Chesser 1991b; Sugg *et al.* 1996; Storz 1999). However, it can be difficult to resolve how these processes interact. Previous studies generally focus at the population level, using biparentally inherited markers only (Chesser 1991b; Pope 1992; Dobson *et al.* 1997, 1998; Storz 1999; Parreira & Chikhi 2015). Here, we show that individual-level fine-scale genetic structure can also be shaped by social processes at uniparentally inherited markers. Furthermore, dispersal can potentially remove any genetic signal of mating behaviour.

While not assessed here, female-biased dispersal should reduce mtDNA structure, whereas male philopatry would reinforce mating systems patterns detected at Y chromosome markers. Additionally, polyandry could potentially bring male and female structure together, reducing the difference in genetic structure between the sexes. While polyandry is relatively rare in mammals (although some cases exist), there are many examples of female-biased dispersal (Dobson 1982; Favre *et al.* 1997; also, see Appendix S1).

A combined marker approach: implications for the agile antechinus

Our findings demonstrate that both dispersal and mating behaviour impact the patterns of fine-scale genetic structure in the agile antechinus, as measured at autosomal, mtDNA and Y chromosome markers. While dispersal has been a primary focus of previous studies of antechinus, simulation findings highlight that patterns of genetic structure can be shaped by a range of processes (Banks *et al.* 2005b; Banks & Peakall 2012; Banks & Lindenmayer 2013). Male-biased dispersal reduced genetic structure in males compared to females across both biparentally and uniparentally inherited markers. Promiscuity also reduced male genetic structure, but only at Y chromosome markers, however, this was obscured by high male dispersal. This suggests that the impact of mating behaviour on genetic structure can only be detected when both sexes are philopatric, which does not occur in the agile antechinus (although many examples exist in other wild populations of small mammals, see Appendix S1).

A combined marker approach: implications for studies of other species

There remains potential to use the combined marker approach to learn about both dispersal and mating behaviour by sampling pre- and post-dispersal individuals, as the level of genetic structure detected can vary dramatically with temporal sampling (Balloux & Lugon-Moulin 2002). While our simulations were parameterised with discrete generations, systems with overlapping generations add new dimensions to spatial genetic patterns, such as inter-generational comparisons (Blyton *et al.* 2015). In a simulation study, Blyton *et al.* (2015) found that as generational overlap increased, spatial genetic structure also increased for both sexes. Therefore, in scenarios of overlapping generations, restricting comparisons of spatial genetic structure to particular groups of

individuals (e.g. adults only or pre- *versus* post-dispersal individuals) will help to link the observed patterns to the underlying process. However, in the semelparous antechinus, fine-scale genetic patterns detected in pre-dispersal individuals will be shaped by mating behaviour (and should reflect patterns shown in our F100/M100 scenario), while post-dispersal individuals should show a clear pattern of male-biased dispersal across all marker types (similar to our F100/M500 scenario). Additionally, our results indicate that it is still possible to detect these patterns when there are different levels of diversity between marker types (Appendix S3).

Comparisons of sex-specific patterns of fine-scale spatial genetic structure at autosomal, mitochondrial and Y chromosome markers, for both pre- and post-dispersal individuals, are expected to be of interest for many species. For example, differences in spatial autocorrelation between the sexes that are congruent across autosomal, mtDNA and Y chromosome markers would indicate dispersal is the predominant driver of fine-scale spatial genetic structure. Alternatively, inconsistent patterns across markers would indicate a mating system influence. If these patterns change between individuals from different age groups (e.g. pouch young or young at foot *versus* adults) then the impact of dispersal and mating behaviour on fine-scale genetic structure could be directly compared and these processes more accurately inferred in wild populations. This is a powerful approach, as detecting the genetic signatures of mating and dispersal independently of each other would allow studies to avoid making assumptions about which processes are shaping these genetic patterns. This is particularly important, given that mammals span the continuum of mating and dispersal strategies.

Implications for other approaches to measuring spatial genetic structure

Here, we employed spatial autocorrelation analysis to quantify the fine-scale, individual by individual spatial genetic patterns arising from different dispersal and mating system scenarios. This approach has the advantage of enabling visualisation of the magnitude and spatial extent of genetic structure at this fine-scale. However, these patterns are also likely to be apparent using population-level statistics. For example, in our simulations the interactive effects of dispersal and mating system variation were also detectable at the population level using an Analysis of Molecular Variance (AMOVA; Excoffier *et al.* 1992;

Peakall *et al.* 1995; Michalakis & Excoffier 1996). Figure 5 shows an infographic of the AMOVA results obtained from an entire simulated landscape (5.6 x 5.6 km, under promiscuity λ= 3 and restricted dispersal), for mtDNA and Y chromosome comparisons of females and males. At the population level, this analysis detected sex-specific differences in genetic structure similar to the patterns shown by spatial autocorrelation analysis, demonstrating that these analyses can be complementary. A key difference is that population-level analyses typically involve the sampling of pre-defined sub-population units (based on spatial scale and location). Thus, it is important to recognise that the spatial scale of sub-population sampling can have a large bearing on the results. In our example, the level of genetic structure detected with AMOVA varied depending on the distance between populations and the spatial distribution of samples.

Other factors shaping genetic patterns

The majority of studies using markers with different modes of inheritance have focused on long term or population-level estimates of gene flow, using F-statistics, estimates of effective population size (N_e) or assignment tests and comparing these metrics among markers (Schubert *et al.* 2011; Nietlisbach *et al.* 2012; Hedrick *et al.* 2013; MacDonald *et al.* 2014; Verkuil *et al.* 2014). However, factors like mutation, genetic drift, bottlenecks, founder effects and selection are strongly influenced by the evolutionary history of a species and shape background levels of genetic diversity (Hedrick 2007; Charlesworth 2009; Banks *et al.* 2013; MacDonald *et al.* 2014). Therefore, when directly comparing patterns among different markers, these factors must be taken into account.

Here, we use an alternative approach, where the comparison is between the sexes rather than between marker types. The patterns are then only compared across markers for congruence, except when comparing mtDNA to the Y chromosome. However, the effective sizes of mtDNA and Y chromosome markers are expected to be equal, as both are haploid and lack recombination (Petit *et al.* 2002). Furthermore, Yannic *et al.* (2012) found that a 100-fold difference in mutation rates between mtDNA and the Y chromosome in their model had negligible effects on their ability to detect sex-biased dispersal using population-level analyses, as mutation rates were small compared to other parameters.

Conclusions

Our computer simulations, initially parameterised for the agile antechinus and extended to represent a broad range of mating and dispersal strategies found in small mammals, revealed that dispersal was the major driver of fine-scale genetic structure across maternally, paternally and biparentally inherited markers. When dispersal was restricted, the mtDNA *versus* Y chromosome comparison was sensitive to variation in mating systems. Three aspects of mating behaviour, promiscuity (multiple sires per litter), polygyny (multiple litters per sire) and reproductive skew, caused changes in the spatial structure of male Y chromosomes compared to female mtDNA that led to patterns similar to those expected under sex-biased dispersal in some cases. Thus caution is required when inferring ecological processes from genetic results. Nonetheless, assessing whether female and male patterns are congruent or different across markers with different modes of inheritance, and whether these patterns change when individuals are sampled at different times, may help disentangle the different ecological and behavioural processes shaping genetic structure within populations.

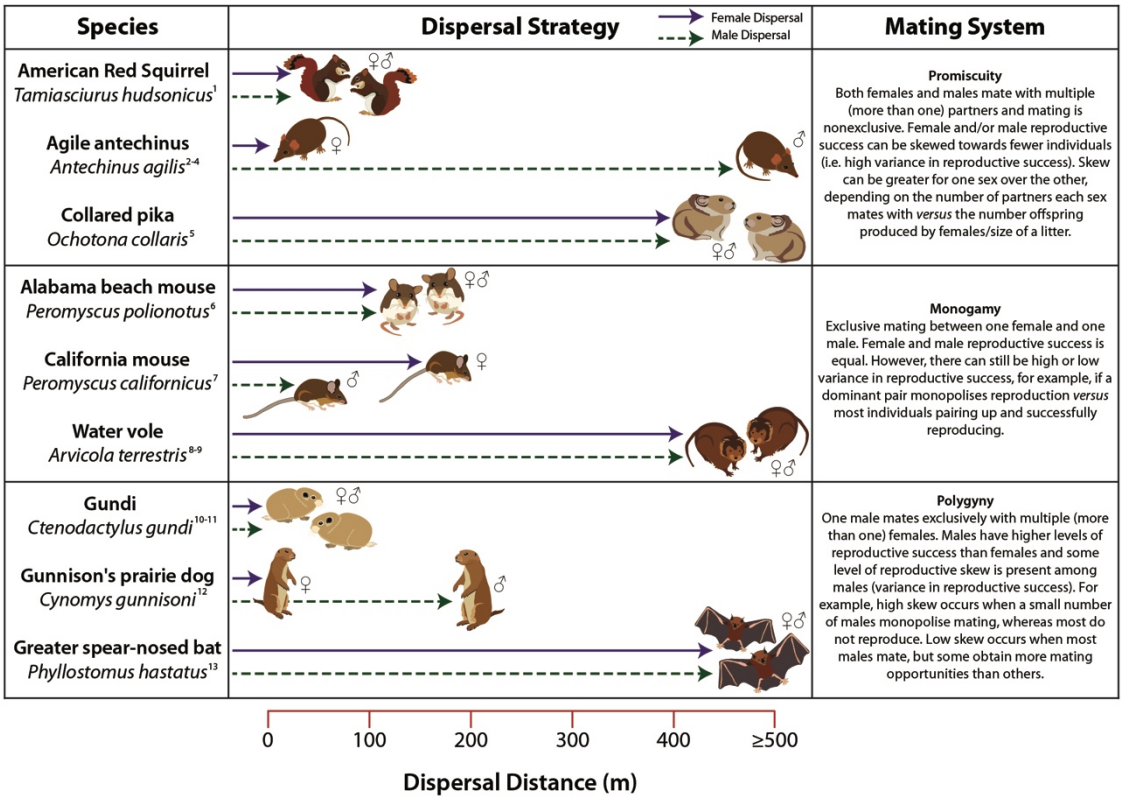

Fig. 1 Mating and dispersal patterns in mammals vary across a continuum, from promiscuity to monogamy, and philopatry to high dispersal (for an extensive list of examples, see Appendix S1). Mating systems can also differ between social mating systems (based on spatial and temporal relationships) compared to genetic mating systems (based on the actual parentage of offspring). Here, we show an example of the variation in mating systems and dispersal patterns across small mammals, over fine-scales (tens – hundreds of meters). We focus on genetic mating systems, with definitions based on the number of mating partners involved in a breeding event, with definitions following Campbell *et al.* (2006) and McEachern *et al.* (2009). Polyandry is not considered in this study, as it is fairly uncommon in mammals (but see Appendix S1 for some examples). All figures were drawn or edited using Adobe Illustrator CC 2014.

Figure References: [1]Larsen & Boutin 1994 [2]Cockburn *et al.* 1985 [3]Kraaijeveld-Smit *et al.* 2002b [4]Banks 2005 [5]Zgurski & Hik 2012 [6]Swilling & Wooten 2002 [7]Ribble 1992 [8]Telfer *et al.* 2003 [9]Aars *et al.* 2006 [10]Nutt 2005 [11]Nutt 2008 [12]Hoogland 1998 [13]McCracken & Bradbury 1981

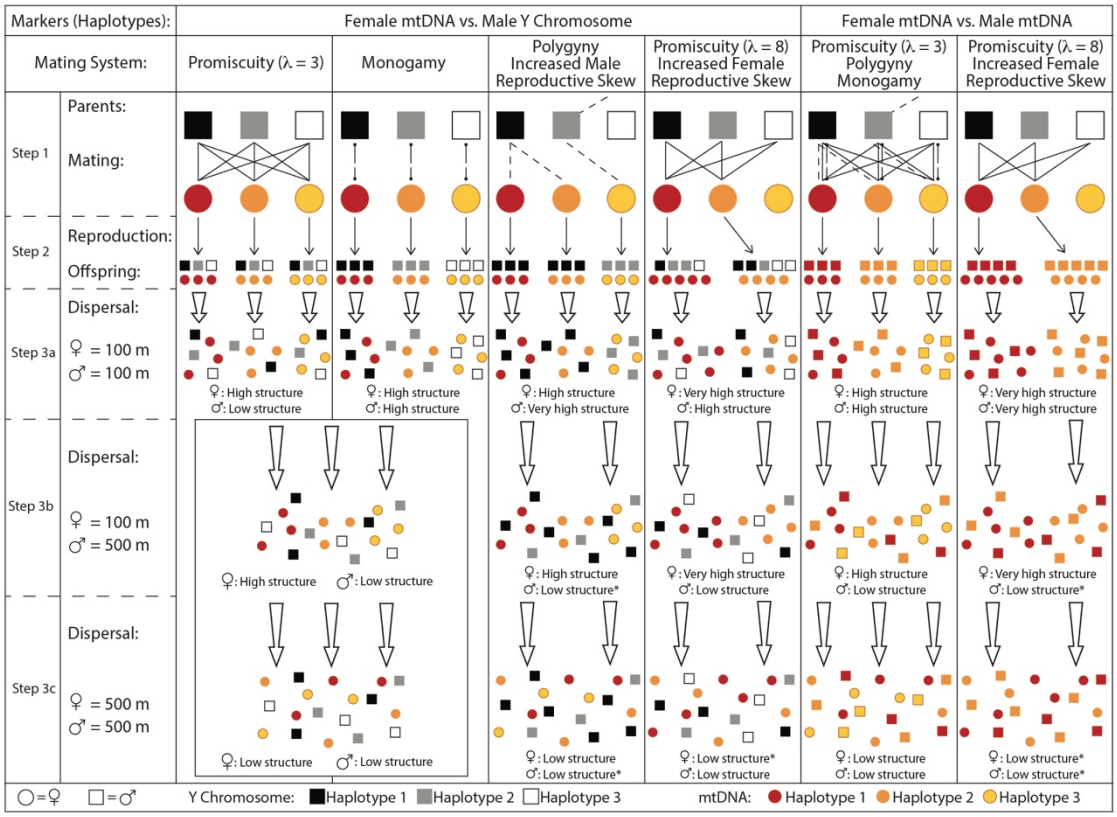

Fig. 2 The impact of mating behaviour and dispersal on fine-scale genetic structure for females and males, at uniparentally inherited markers. *Step 1:* Females (circles) and males (squares) involved in mating are indicated by the solid (promiscuity), broken (monogamy) and dashed (polygyny) lines. **Female mtDNA *vs.* male Y chromosome:** *Step 2:* Female offspring share the same mtDNA haplotype as their sisters within a litter, but are genetically different to females in other litters. Conversely, male genetic structure at Y chromosome markers varies depending on the mating system. *Step 3a:* When dispersal is restricted in both sexes, the patterns developed under each mating system are maintained. *Step 3b:* Under male-biased dispersal, female structure remains high, whereas male dispersal randomly distributes Y chromosome haplotypes throughout the population. *Step 3c:* High dispersal in both sexes randomly distributes mtDNA and Y chromosome haplotypes throughout the population. **Female mtDNA *vs.* male mtDNA:** *Step 2:* No difference in genetic structure is detected when comparing both sexes at mtDNA markers. *Steps 3a-c:* Dispersal reduces genetic structure at mtDNA markers. Female skew increases the overall magnitude of genetic structure, but this impacts both sexes equally (*exceptions: here, only three haplotypes are represented, creating high levels of genetic structure in these examples. With more individuals in the population, dispersal would introduce more haplotype variation and this structure would also likely be reduced).

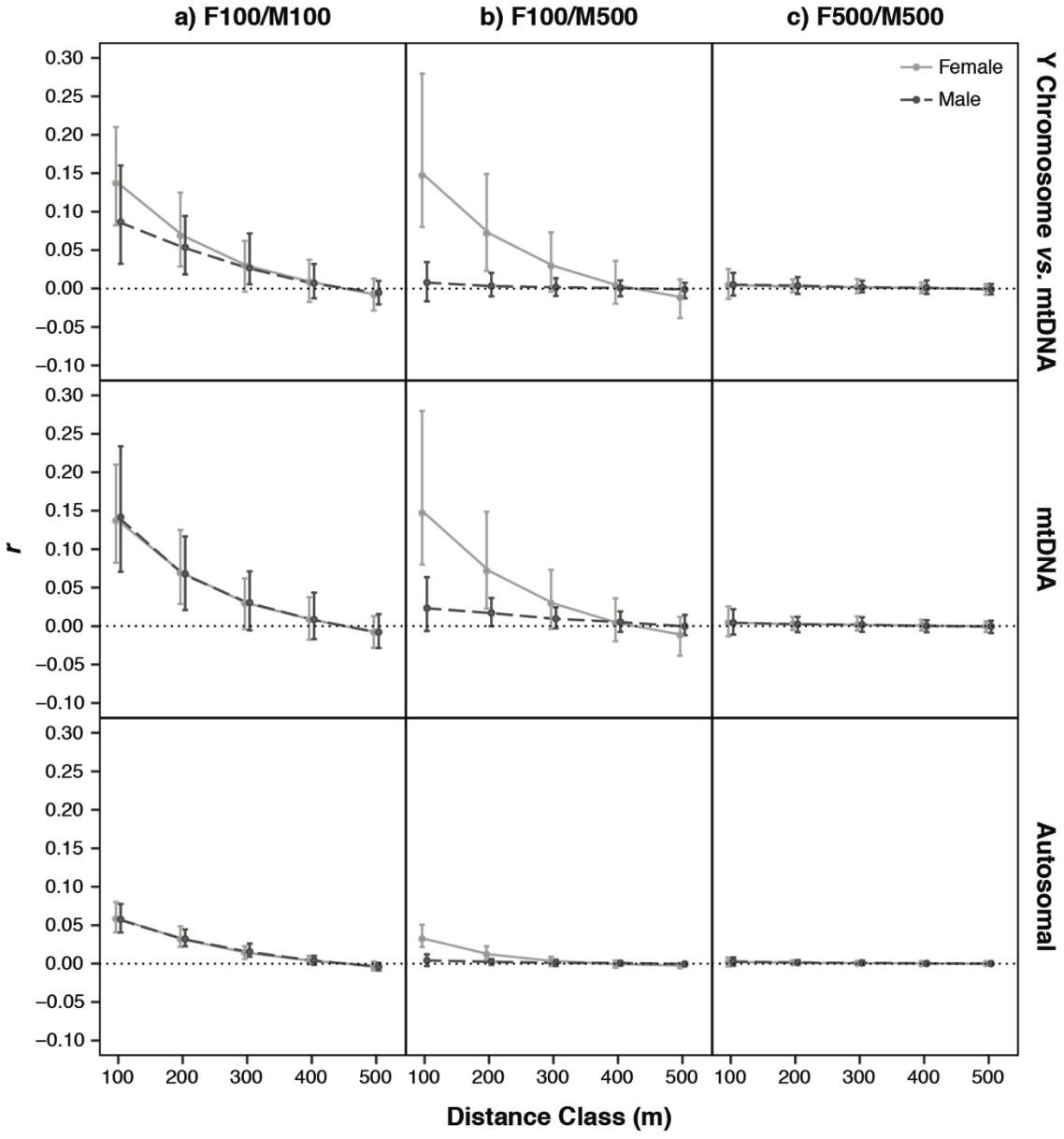

Fig. 3 Correlograms for females and males with mean autocorrelation *r* values generated over 100 simulations, at the 100th generations (n = 500), across autosomal, mtDNA and Y chromosome markers. Simulations represent restricted dispersal (column a: F100/M100), male-biased dispersal (column b: F100/M500) and high dispersal (column c: F500/M500), for a promiscuous mating system (λ = 3). Error bars around the autocorrelation *r* values represent the 2.5 – 97.5 percentiles of the distribution of *r* values across simulations. Figures were prepared in R 3.2.2 (R Core Team 2015). Correlograms were generated in ggplot2 (Wickham 2009).

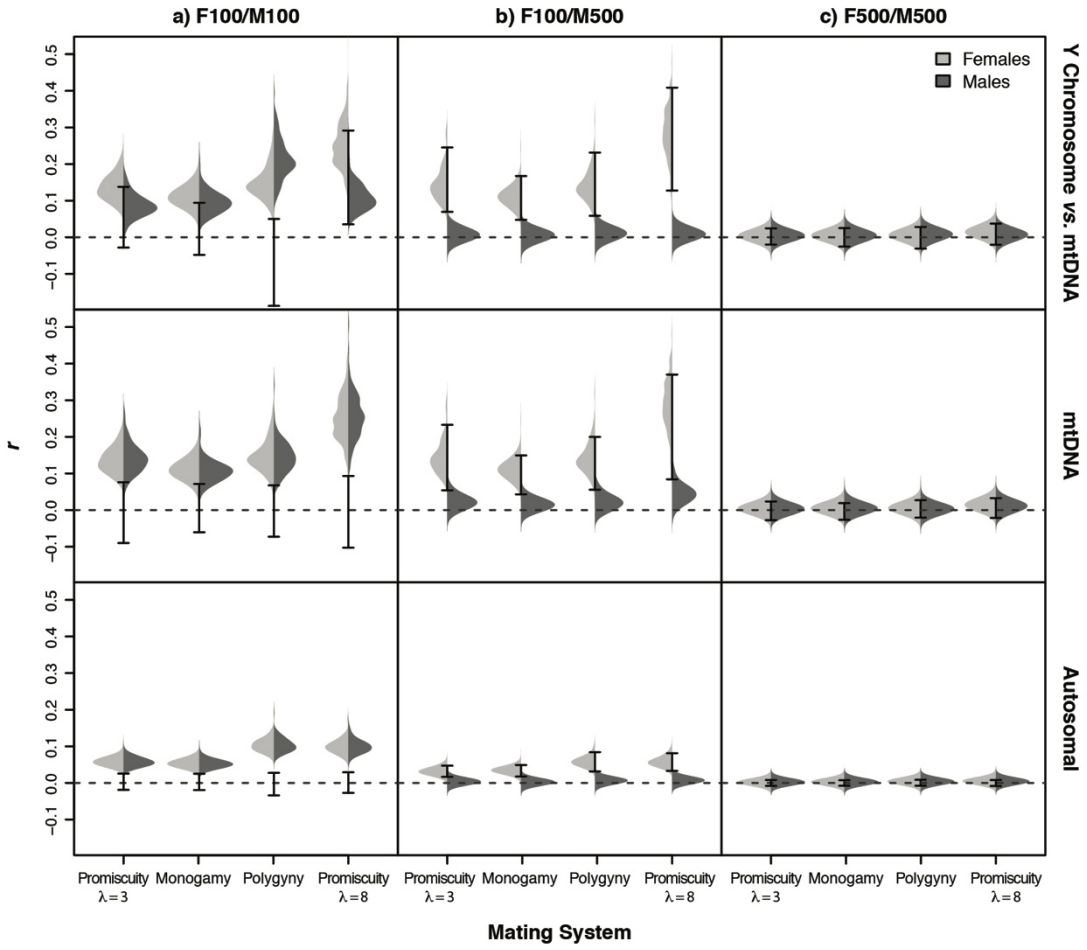

Fig. 4 Back to back bean plots showing female and male distributions of simulated spatial autocorrelation *r* values in the first distance class (0-100 m) across autosomal, mtDNA and Y chromosome markers. Different dispersal scenarios are represented in panel columns [a) restricted dispersal, b) male-biased dispersal and c) high dispersal]. Mating systems and levels of reproductive skew are shown on the *x* axis. The vertical bars in the centre of each bean plot show the 2.5 – 97.5 percentiles of the difference in *r* value distributions between females and males ($r_{females}$ - r_{males}). When the vertical bars shift towards positive values, females generally show greater structure than males, while a negative direction means that male structure is generally greater than that of females (for the significance of individual simulations see Appendix S10) (R package: Bean plot, Kampstra 2008).

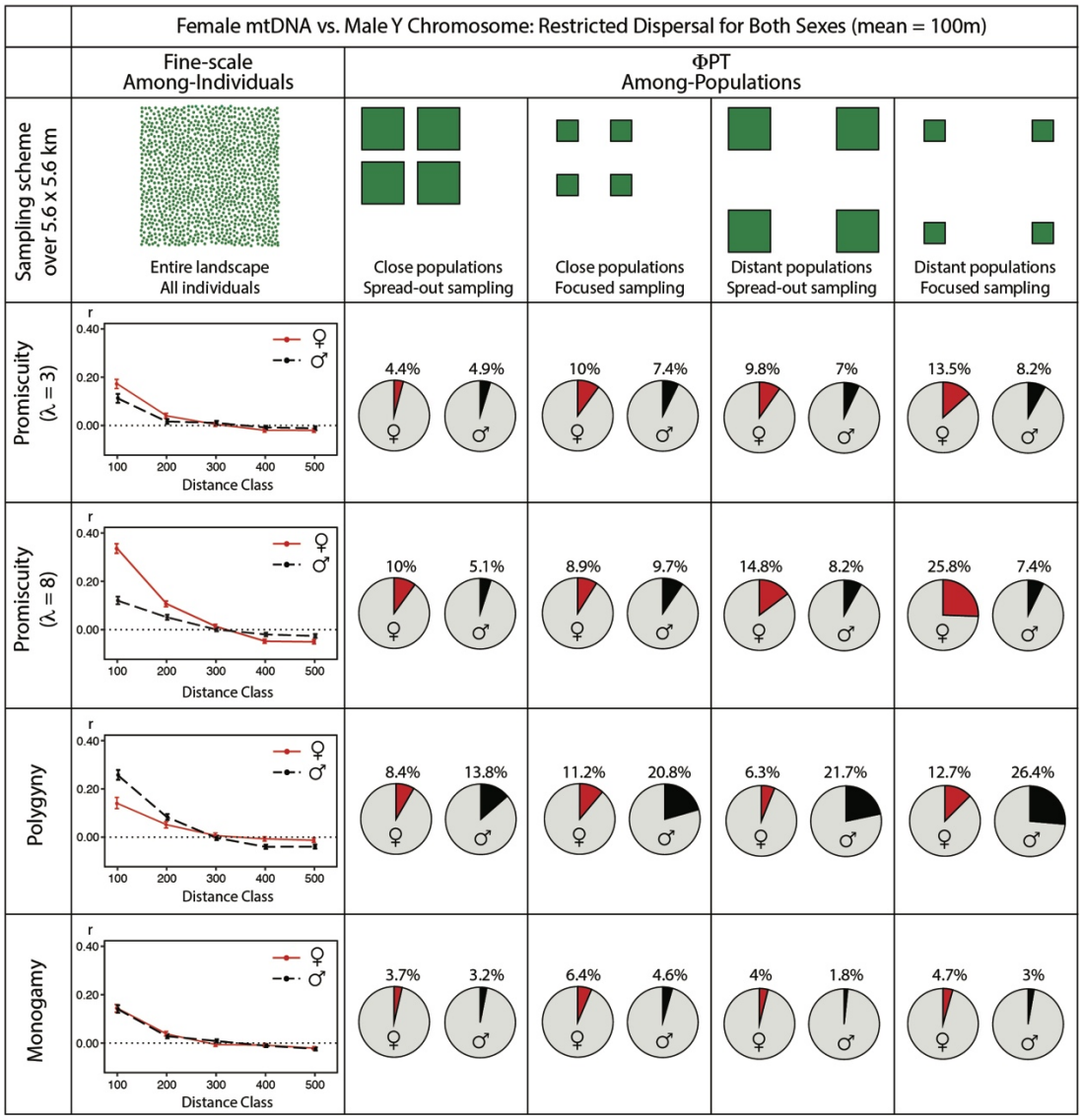

Fig. 5 A visual demonstration of the concordance between individual-level *versus* population-level analyses (multilocus spatial autocorrelation analysis *vs.* an Analysis of Molecular Variance - AMOVA). Restricted dispersal (F100/M100) was simulated under each mating system. Different groups of individuals were then analysed within the same, single simulation (for each mating system). **Spatial autocorrelation analysis:** This analysis was performed on individuals spread across the entire landscape. Significant differences in the level of fine-scale genetic structure were detected between the sexes for all mating systems except monogamy (in the first distance class). **AMOVA:** This analysis was performed over four "populations", defined using different sampling schemes (with each population made up of a random subset of 125 individuals). The highlighted section of the pie chart represents the percentage of among population differentiation (ΦPT, an analogue of F_{ST}). AMOVA results reflect spatial autocorrelation patterns. However, the level of population structure (as detected by AMOVA) varies depending on how populations are defined across the landscape and how individuals are sampled. (Analyses were performed in GenAlEx 6.5: Peakall & Smouse 2006, 2012)

Table 1 Means and 2.5 – 97.5 percentiles of female and male *r* values under all simulation scenarios (dispersal and mating behaviour), for autosomal, mtDNA and Y chromosome markers.

Marker	Dispersal	Mating System	Female *r* mean ± SE	Male *r* mean ± SE	Female *r* 2.5 – 97.5 Percentiles	Male *r* 2.5 – 97.5 Percentiles
Autosomal	F100M100	Monogamy	0.054 ± 0.001	0.053 ± 0.001	0.038 to 0.074	0.036 to 0.073
		Polygyny	0.103 ± 0.002	0.104 ± 0.002	0.077 to 0.141	0.074 to 0.141
		Promiscuity (λ=3)	0.058 ± 0.001	0.057 ± 0.001	0.04 to 0.08	0.04 to 0.078
		Promiscuity (λ=8)	0.1 ± 0.002	0.1 ± 0.002	0.068 to 0.138	0.07 to 0.143
	F100M500	Monogamy	0.035 ± 0.001	0.003 ± 0	0.023 to 0.051	-0.004 to 0.01
		Polygyny	0.059 ± 0.001	0.007 ± 0	0.039 to 0.088	-0.003 to 0.016
		Promiscuity (λ=3)	0.033 ± 0.001	0.004 ± 0	0.021 to 0.051	-0.002 to 0.012
		Promiscuity (λ=8)	0.058 ± 0.001	0.007 ± 0	0.039 to 0.087	-0.001 to 0.016
	F500M500	Monogamy	0.003 ± 0	0.003 ± 0	-0.003 to 0.009	-0.002 to 0.008
		Polygyny	0.004 ± 0	0.004 ± 0	-0.001 to 0.011	0 to 0.009
		Promiscuity (λ=3)	0.002 ± 0	0.003 ± 0	-0.004 to 0.008	-0.003 to 0.008
		Promiscuity (λ=8)	0.005 ± 0	0.005 ± 0	0 to 0.011	-0.002 to 0.011
mtDNA	F100M100	Monogamy	0.111 ± 0.003	0.107 ± 0.003	0.06 to 0.17	0.06 to 0.181
		Polygyny	0.148 ± 0.004	0.145 ± 0.004	0.079 to 0.246	0.072 to 0.224
		Promiscuity (λ=3)	0.137 ± 0.003	0.142 ± 0.004	0.082 to 0.21	0.071 to 0.234
		Promiscuity (λ=8)	0.255 ± 0.007	0.255 ± 0.007	0.142 to 0.4	0.135 to 0.382
	F100M500	Monogamy	0.113 ± 0.003	0.016 ± 0.001	0.066 to 0.169	-0.002 to 0.039
		Polygyny	0.142 ± 0.004	0.023 ± 0.002	0.074 to 0.235	-0.008 to 0.065
		Promiscuity (λ=3)	0.15 ± 0.005	0.026 ± 0.002	0.073 to 0.239	-0.003 to 0.055
		Promiscuity (λ=8)	0.283 ± 0.007	0.046 ± 0.003	0.133 to 0.413	0.004 to 0.106
	F500M500	Monogamy	0.005 ± 0.001	0.005 ± 0.001	-0.01 to 0.02	-0.011 to 0.024
		Polygyny	0.006 ± 0.001	0.004 ± 0.001	-0.013 to 0.024	-0.015 to 0.019
		Promiscuity (λ=3)	0.004 ± 0.001	0.004 ± 0.001	-0.014 to 0.026	-0.011 to 0.022
		Promiscuity (λ=8)	0.015 ± 0.001	0.011 ± 0.001	-0.004 to 0.039	-0.011 to 0.034
mtDNA vs. Y Chromosome	F100M100	Monogamy	0.111 ± 0.003	0.096 ± 0.003	0.06 to 0.17	0.051 to 0.152
		Polygyny	0.148 ± 0.004	0.214 ± 0.006	0.079 to 0.246	0.118 to 0.336
		Promiscuity (λ=3)	0.137 ± 0.003	0.087 ± 0.003	0.082 to 0.21	0.032 to 0.16
		Promiscuity (λ=8)	0.255 ± 0.007	0.111 ± 0.004	0.142 to 0.4	0.055 to 0.194
	F100M500	Monogamy	0.113 ± 0.003	0.006 ± 0.001	0.066 to 0.169	-0.02 to 0.028
		Polygyny	0.142 ± 0.004	0.011 ± 0.001	0.074 to 0.235	-0.015 to 0.037
		Promiscuity (λ=3)	0.15 ± 0.005	0.005 ± 0.001	0.073 to 0.239	-0.017 to 0.028
		Promiscuity (λ=8)	0.283 ± 0.007	0.009 ± 0.001	0.133 to 0.413	-0.014 to 0.036
	F500M500	Monogamy	0.005 ± 0.001	0.005 ± 0.001	-0.01 to 0.02	-0.014 to 0.026
		Polygyny	0.006 ± 0.001	0.008 ± 0.001	-0.013 to 0.024	-0.012 to 0.028
		Promiscuity (λ=3)	0.004 ± 0.001	0.005 ± 0.001	-0.014 to 0.026	-0.009 to 0.021
		Promiscuity (λ=8)	0.015 ± 0.001	0.007 ± 0.001	-0.004 to 0.039	-0.014 to 0.023

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Appendix

Appendix S1: Mammalian mating systems and dispersal strategies over fine-scales (tens to hundreds of meters): examples from the literature

Species	Common name	Genetic mating system	Mating system caveats	Dispersal strategy*	Dispersal caveats	Dispersal distance (m)	References
Tamiasciurus hudsonicus	American Red Squirrel	• Promiscuity	N/A	• Restricted dispersal • No sex-bias	All offspring remained within potential contact of natal territory	Mean: ♀♂ = 88.6	Larsen & Boutin 1994
Dipodomys spectabilis	Banner-tailed kangaroo rat	• Promiscuity	Socially polygynandrous.	• Restricted dispersal • Female-biased dispersal	Dispersal is density dependent in females. Proportion of females breeding within 50 m of natal mound significantly decreased with density (86% to 59%).	Median: ♀♂: High density= 13-16 Low density= 30	Jones *et al.* 1988; Busch *et al.* 2009; Steinwald *et al.* 2013
Antechinus agilis	Agile antechinus	• Promiscuity	N/A	• Male-biased dispersal	N/A	Mean: ♀ = 40 ♂ = 1250	Cockburn *et al.* 1985: Kraaijeveld-Smit *et al.* 2002; Banks 2005
Antechinus stuartii	Brown antechinus	• Promiscuity	N/A	• Male-biased dispersal	N/A	Mean: ♀ = 57 ♂ = 387	Lazenby-Cohen & Cockburn 1988; Fisher 2005; Banks *et al.* 2011
Ochotona collaris	Collared pika	• Promiscuity	Socially polygynandrous.	• High dispersal • No sex-bias	N/A	Mean: ♀♂ = 536	Zgurski & Hik 2012
Sciurus vulgaris	Eurasian red squirrel	• Promiscuity	N/A	• High dispersal • No sex-bias	N/A	Mean: ♀♂ = 1014	Wauters *et al.* 2011
Peromyscus polionotus ammobates	Alabama beach mouse	• Monogamy	N/A	• Restricted dispersal	55% of female and male subadults remained philopatric.	Mean: ♀♂ = 160.2	Swilling & Wooten 2002
Microtus ochrogaster	Prairie vole	• Monogamy • Extra-pair paternity	Varies with ecological conditions. Monogamy varied from 72% *vs.* 39% of individuals across two populations.	• Restricted dispersal	The proportion philopatric individuals varies with population density, habitat quality, and adult sex ratio. Has been shown to vary from 75% to 92%.	Mean: ♀♂ = 30	Getz 1997; Keane *et al.* 2017

*continued over page

Species	Common name	Genetic mating system	Mating system caveats	• Dispersal strategy	Dispersal caveats	Dispersal distance (m)	References
Fukomys anselli	Ansell's mole-rat	Monogamy	Reproduction restricted to dominant pair (reproductive success highly skewed).	Restricted dispersal	Dispersal may vary depending on environmental conditions.	♀♂ <200	Patzenhauerová *et al.* 2013
Hypogeomys antimena	Malagasy giant jumping rat	• Monogamy	N/A	• Mild male-biased dispersal	Dispersal dropped to 0 m in both sexes after a population crash.	Mean: ♀ = 181 ♂ = 226	Sommer 2003a; b
Peromyscus californicus	California mouse	• Monogamy	N/A	• Female-biased dispersal	48% of males remained philopatric, compared to 29% of females.	Mean: ♀ = 155 ♂ = 70	Ribble 1992
Arvicola terrestris	Water vole	• Monogamy	Level of monogamy varies with habitat fragmentation.	• High dispersal • No sex-bias	N/A	Median: ♀ = 930 ♂ = 1030	Telfer et al. 2003; Aars et al. 2006
Crocidura russula	Greater white-toothed shrew	• Monogamy • Polygyny	Both monogamy and polygyny have been shown to occur (within a single population).	• Female biased-dispersal • Restricted dispersal	Seasonal-bias in dispersal (only first litter disperse). Dispersal distance indirectly estimated through modelling. Males are highly philopatric and settle on or adjacent to natal territory (territory= 100m2)	Mean: ♀ = 800 ♂ = ~100	Favre et al. 1997; Bouteiller & Perrin 2000; Jaquiéry et al. 2008
Ctenodactylus gundi	Gundi	• Polygyny	N/A	• Restricted dispersal • Possible male-biased dispersal	Dispersal is plastic and can vary with environmental conditions. Mostly philopatric, although possible male-bias in individuals that did disperse (estimated to have occurred across 1.8-2.2 groups - potentially underestimated).	High levels of philopatry found in both sexes: ♀ = 80-85% ♂ = 64-85%	Nutt 2005, 2008
Ctenomys talarum	Talar tuco-tuco	• Polygyny	Degree of polygyny varies with population density.	• Male-biased dispersal	Dispersal inferred from genetic data (distance between individuals that showed high relatedness).	Mean: ♀ = ~20m ♂ = ~60-80	Zenuto et al. 1999; Cutrera et al. 2005
Capreolus capreolus	Roe deer	• Polygyny (low-level)	N/A	• High dispersal • No sex-bias	N/A	Mean: ♀♂ = 1062 – 2124	Gaillard *et al.* 2008; Vanpé *et al.* 2008

**continued over page*

Species	Common name	Genetic mating system	Mating system caveats	• Dispersal strategy	Dispersal caveats	Dispersal distance (m)	References
Phyllostomus hastatus	Greater spear-nosed bat	• Polygyny	Form harems with one adult male. Male reproductive success highly skewed (harem males sire >50 offspring, whereas most males sire 0).	• High dispersal • No sex-bias	Females form stable groups within harems and are a genetically random sample of the population (unrelated).	Juvenile ♀♂ disperse from natal harem to form another harem within the same cave (~ <1km) or in a different colony (~ >1km)	McCracken & Bradbury 1981
Lophostoma silvicolum	Neotropical bat	• Polygyny	N/A	• Random • No sex-bias	Dispersal inferred from genetic data. Dispersal mainly influenced by availability of suitable territory.	♀♂ = random	Dechmann *et al.* 2005, 2007; Dechmann & Kerth 2008
Cryptomys hottentotus hottentotus	Common mole-rat	• Polyandry • Polygyny	Within colony, reproduction restricted to a dominant pair, but extra pair paternity can occur (reproductive success is highly skewed). Sometimes, polygyny can also occur.	• Restricted dispersal • Possible male-biased dispersal	Dispersal rates can vary depending on group size and environmental conditions. Most individuals are philopatric (remain in natal colony). Some subordinates disperse between colonies in optimal conditions	♀ = ~<100m (most likely due to ecological constraints of burrowing)	Spinks *et al.* 2000; Bishop *et al.* 2004
Saguinus fuscicollis	Saddle-back tamarins	• Polyandry • Monogamy • Polygyny	Varies from group to group, depending on the number of helpers. Polyandry and monogamy most common.	• Possible male-biased dispersal	Usually dispersed to neighbouring territory.	More ♂ were immigrants than ♀ (~>100m)	Goldizen 1987; Goldizen *et al.* 1996

* We defined restricted dispersal as settling on (philopatry) or adjacent to the natal territory

Appendix S1 References

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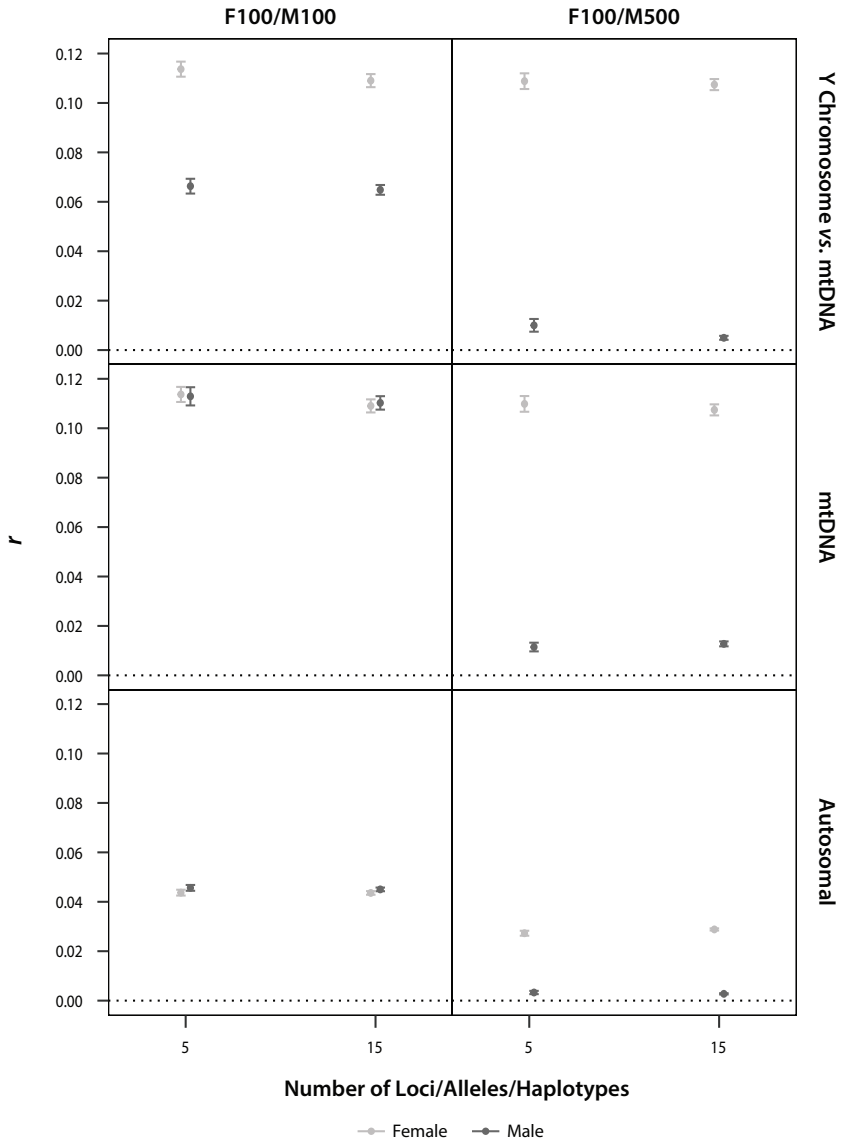
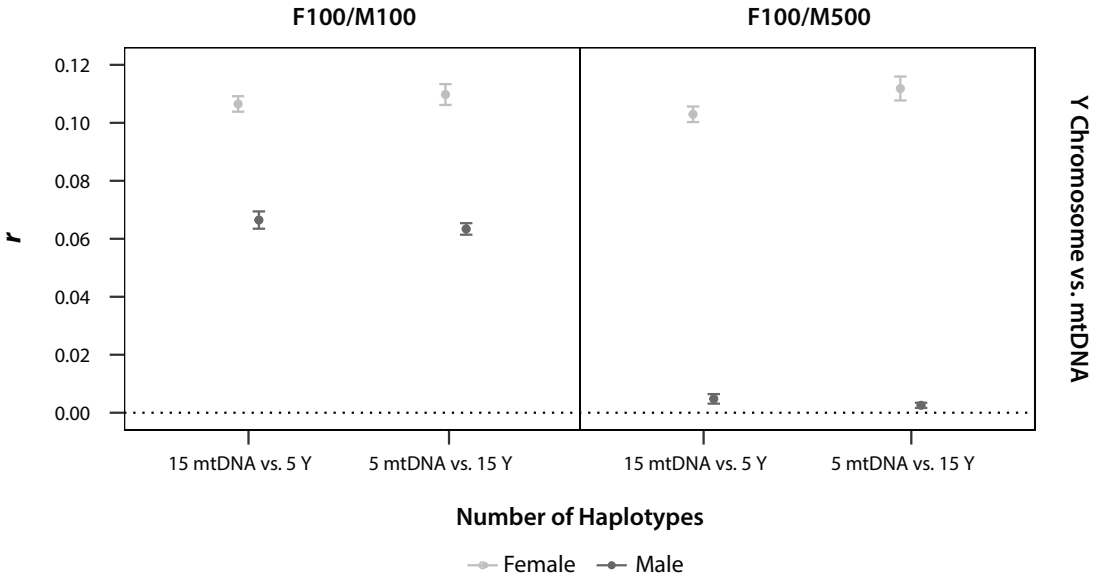
Appendix S2



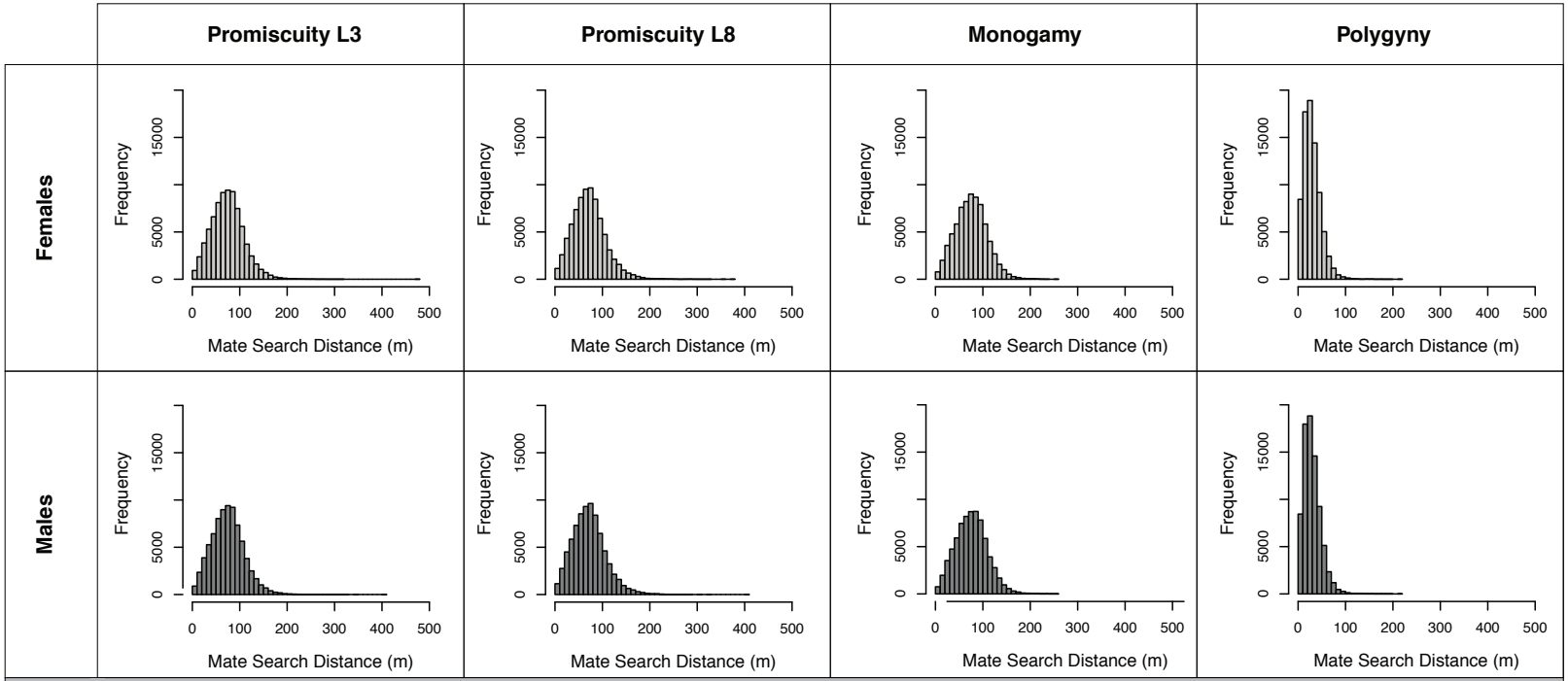
Mean autocorrelation *r* values generated from 100 simulations, shown for males and females in the first distance class (0-100 m) for a promiscuous mating system (λ = 3). The effect of varying the number of loci, alleles and haplotypes on the mean autocorrelation r value is shown under restricted dispersal and sex-biased dispersal for autosomal, mtDNA and Y chromosome markers. Error bars around the autocorrelation r values represent the standard error around the mean. In general, the mean r values do not change substantially as the number of loci, alleles and haplotypes increase. However, the standard error decreases slightly when more markers are used. This means that spatial autocorrelation is fairly robust to differences in diversity among marker types. However, the sensitivity of spatial autocorrelation to detect significant positive genetic structure, or significant differences in structure among the sexes may be impacted.

Appendix S3



Mean autocorrelation *r* values generated from 100 simulations, shown for males and females in the first distance class (0-100 m) under a promiscuous mating system (λ = 3). The effect of having a different number of Y chromosome versus mtDNA haplotypes on the mean autocorrelation r value is shown under restricted dispersal and sex-biased dispersal. Error bars around the autocorrelation r values represent the standard error around the mean. Again, comparing different numbers of haplotypes between males and females at Y chromosome versus mtDNA markers does not substantially change the mean r values and overall pattern of fine-scale genetic structure among the sexes. However, the standard error decreases slightly with more haplotypes, thus the power to detect differences between the sexes may be impacted.

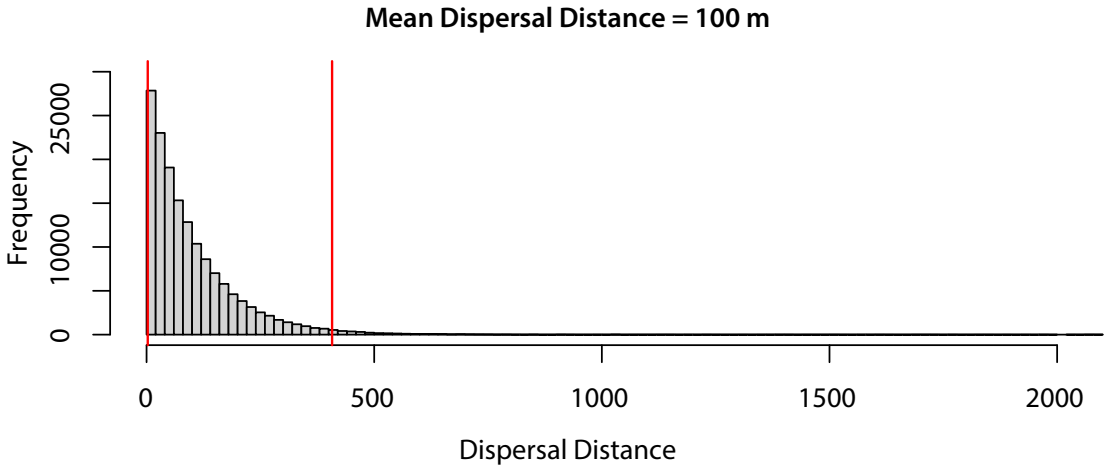
Appendix S4

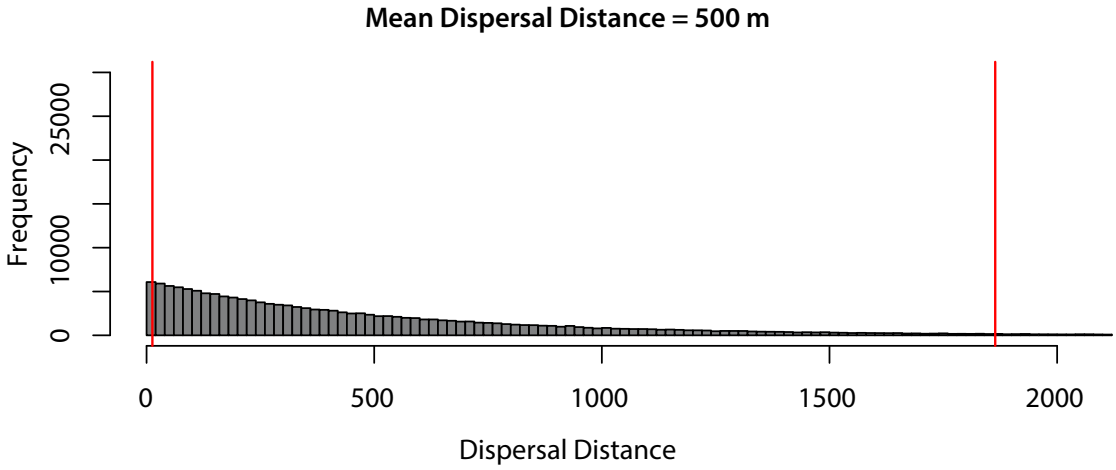


Sex	Females	Males	Females	Males	Females	Males	Females	Males
Mean	75.06	75.36	72.62	72.66	76.01	76.27	30.08	30.08
Standard Error	0.12	0.12	0.12	0.13	0.12	0.12	0.06	0.06
Variance	1157.65	1171.82	1206.39	1239.67	1076.31	1087.53	317.48	315.61
2.5 Percentile	16	16	14	14	16	16	5	5
97.5 Percentile	149	149	151	152	144	144	72	72

Distance searched to find a mate by females and males under each mating system. Distributions and summary statistics across 10 simulations, for all individuals in the simulation landscape (n = 157000).

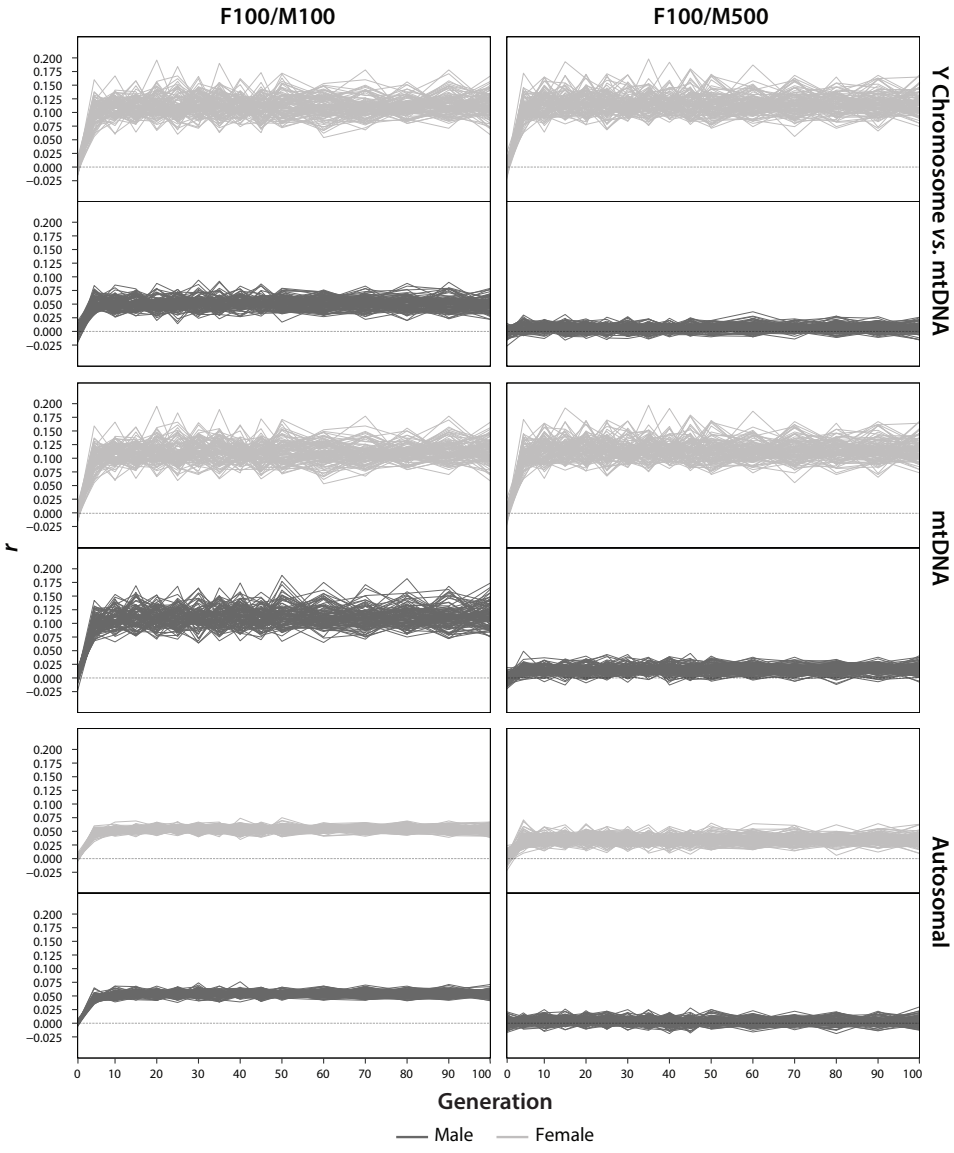
Appendix S5





Dispersal distances for all individuals in the simulation landscape, across 10 simulations (n = 157000), when the mean dispersal distance was set to 100 meters versus 500 meters. Dispersal distances follow an exponential distribution and red lines indicate the 2.5 and 97.5 percentiles.

Appendix S6



Autocorrelation *r* values for 100 simulations, shown for females and males in the first distance class (0-100m) over 100 generations. Simulations were carried out under restricted dispersal (F100/M100) and sex-biased dispersal (F100/M500) for a promiscuous mating system ($\lambda = 3$) across autosomal, mtDNA and Y chromosome markers. Each line represents a single simulation output.

Appendix S7

Mean, variance and standard error for the number of offspring produced per female and male under each mating system, across 100 simulations ($n_{females}$ = 785000, n_{males} = 785000). Statistics were calculated based on: 1) only those individuals that successfully reproduced and 2) all individuals in the simulation landscape (including the individuals that produced 0 offspring).

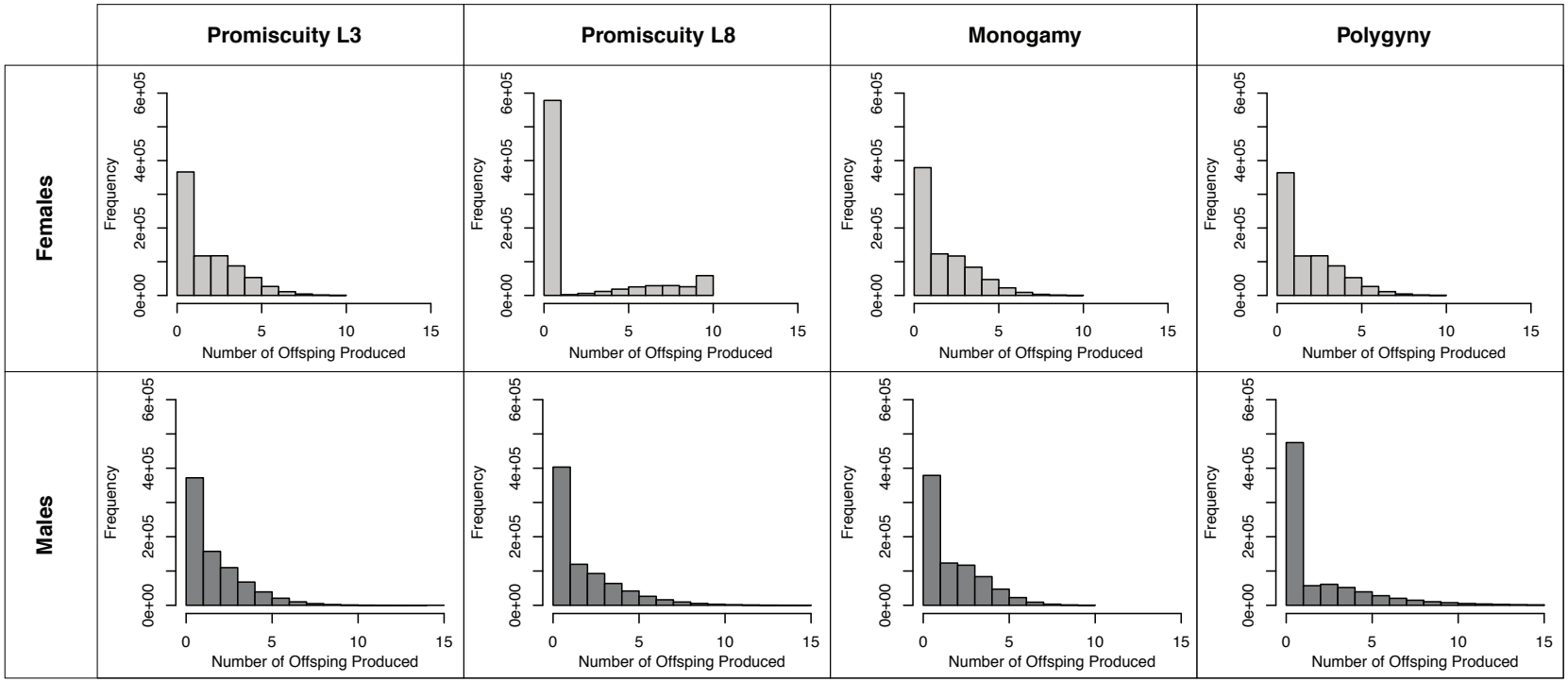
		Successful Individuals Only		All Individuals (Including Unsuccessful)	
Mating System	**Summary Statistic**	**Females**	**Males**	**Females**	**Males**
Promiscuity L3	**Mean**	3.15	2.61	2.00	2.00
	Variance	2.65	2.82	3.99	3.38
	Standard Error	0.00	0.00	0.00	0.00
Promiscuity L8	**Mean**	7.58	3.13	2.00	2.00
	Variance	4.67	4.42	12.40	5.09
	Standard Error	0.00	0.00	0.00	0.00
Monogamy	**Mean**	3.02	3.02	1.90	1.90
	Variance	2.48	2.48	3.69	3.69
	Standard Error	0.00	0.00	0.00	0.00
Polygyny	**Mean**	3.16	4.55	2.01	2.01
	Variance	2.65	9.43	3.99	9.26
	Standard Error	0.00	0.01	0.00	0.00

Appendix S8

Mean, variance and standard error for the number of parents under each mating system, across 100 simulations ($n_{females}$ = 785000, n_{males} = 785000), as well as the percent of individuals that did/did not reproduce.

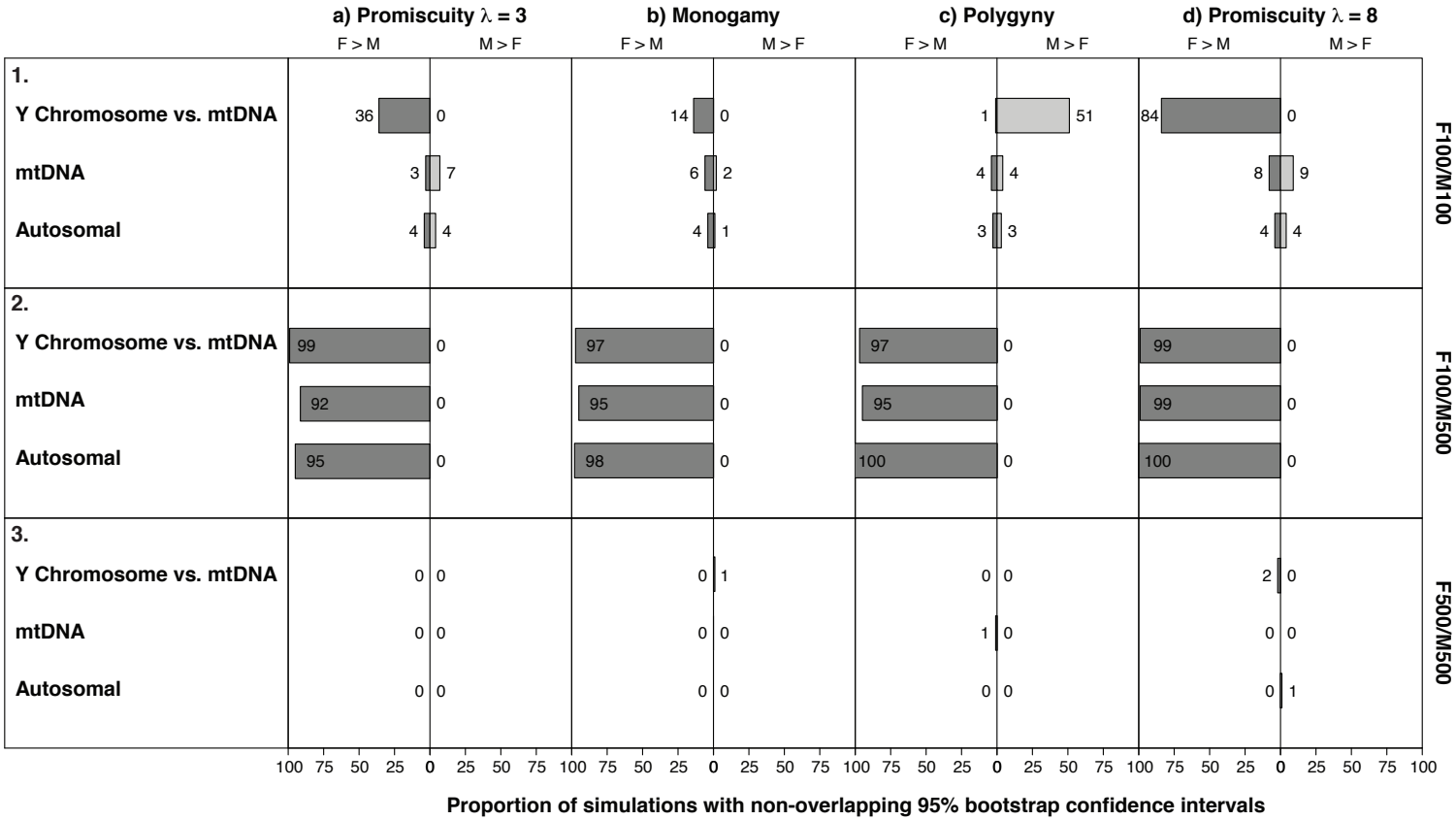
Mating System	Statistic	Females	Males
Promiscuity L3	Mean	4978.31	6014.34
	Variance	1258.44	1834.79
	Standard Error	3.55	4.28
	% of individuals that successfully reproduced	31.71	38.31
	% of individuals that did not reproduce	68.29	61.69
Promiscuity L8	Mean	2070.47	5008.00
	Variance	145.20	2102.67
	Standard Error	1.20	4.59
	% of individuals that successfully reproduced	13.19	31.90
	% of individuals that did not reproduce	86.81	68.10
Monogamy	Mean	4933.92	4933.92
	Variance	1189.61	1189.61
	Standard Error	3.45	3.45
	% of individuals that successfully reproduced	31.43	31.43
	% of individuals that did not reproduce	68.57	68.57
Polygyny	Mean	4974.53	3450.76
	Variance	1521.20	1108.08
	Standard Error	3.90	3.33
	% of individuals that successfully reproduced	31.68	21.98
	% of individuals that did not reproduce	68.32	78.02

Appendix S9



Number of offspring produced by females and males under each mating system. Distributions across 100 simulations, including all individuals in the simulation landscape (n = 1570000).

Appendix S10



Pyramid plots showing the proportion of simulations where female and male 95% bootstrap confidence intervals did not overlap (indicating a significant difference in fine-scale genetic structure among the sexes). Mating systems and reproductive skew are shown in the panel columns a) – d) and dispersal scenarios are shown for autosomal, mtDNA and Y chromosome markers across the panel rows [1. restricted dispersal, 2. male-biased dispersal and 3. high dispersal]. F > M represents simulations where female structure was significantly greater than male structure, while M > F represents males having significantly greater structure than females (R package: Lemon J (2006) Plotrix: a package in the red light district of R. *R-News*, **6**, 8–12.)

Chapter 3

Evaluating population genetic patterns across molecular markers, alignment methods and SNP filtering strategies in a native Australian rodent

Abstract

Genetic data can be a powerful addition to studies investigating ecological and biological processes in animal populations. A range of different molecular markers are available for such research, including those with different inheritance modes. Furthermore, high throughput sequencing data is becoming increasingly accessible, with common methods involving reduced representation Next-Generation Sequencing. These methods have the potential to identify up to hundreds of thousands of Single Nucleotide Polymorphisms (SNPs). However, stringent filtering is required if we are to have the confidence in individual genotypes that is required for fine-scale population genetic analyses.

We explored an empirical dataset, from a native Australian rodent, comprising a range of different marker types, including SNPs genotyped using different bioinformatic approaches. Using a number of population genetic analyses typical in studies of dispersal, we compared patterns of genetic diversity and spatial genetic structure across SNPs, microsatellites, mitochondria and the Y chromosome. We also investigated how different bioinformatic pipelines and filtering criteria influenced the resulting population genetic patterns.

We found that both bioinformatic pipelines and filtering can impact population genetic results. Estimates of within population diversity (observed heterozygosity and F_{IS}) varied between different bioinformatic pipelines and filtering strategies. In contrast, differentiation among populations (F_{ST} and its analogues) and distance-based metrics were robust. These results demonstrate the importance of understanding how methodological decisions can impact biological inference from genetic data, and the value of comparing patterns across markers with different modes of inheritance.

Introduction

The increasing availability of cost-effective, commercially available Next-Generation Sequencing (NGS) data has transformed the application of genetics in the field of ecology (Allendorf et al. 2010, Funk et al. 2012). Fundamental questions about dispersal and population connectivity can now be investigated more effectively through the use of genetic data with relative ease (Meirmans and Hedrick 2011). However, decisions about marker choice, bioinformatic pipelines and filtering can be overwhelming for experts and non-experts alike. It is crucial that researchers understand the strengths and limitations of adopting such techniques, as these decisions can impact the outcomes of downstream analyses and thus have the potential to change the biological conclusions drawn from such data (Torkamaneh et al. 2016, Shafer et al. 2017).

Autosomal microsatellites have been the mainstream marker in ecological research for decades, however, they have largely been replaced by Single Nucleotide Polymorphisms (SNPs) (Maguire et al. 2002, Steiner et al. 2013). Microsatellite markers are multi-allelic, making them particularly efficient for exploring population genetic questions (Hedrick 1999, Maguire et al. 2002). SNPs are typically bi-allelic, meaning that the information content of each locus is low. However, NGS studies make up for low polymorphism by using thousands to hundreds of thousands of SNP loci across the genome (Cappa et al. 2016). SNPs can be used to tackle additional questions not possible using microsatellites, such as identifying adaptive genetic variation (Allendorf et al. 2010). However, it is important to understand if and how results differ between SNPs and microsatellite markers for population genetic analyses.

Microsatellite markers are specifically targeted through the use of primers, whereas SNPs can be obtained through a number of NGS techniques. Among the most popular for ecological studies are reduced representation approaches (Torkamaneh et al. 2016). Reduced representation approaches (including Diversity Arrays Technology sequencing [DArTseq], genotyping by sequencing [GBS], and restriction site associated DNA sequencing [RAD-Seq]) work by first randomly shearing the DNA into different size fragments by using one or more restriction enzymes. These fragments are tagged with

adapters (for sequencing on NGS platforms) and unique barcodes (for individual identification, allowing samples to be multiplexed), then amplified through PCR (Andrews et al. 2016). Finally, the number of loci is reduced through a size selection step. After sequencing, the raw data are passed through quality control, aligned and screened for SNPs, moving from the wet lab into the realm of bioinformatics.

Bioinformatic pipelines for SNP calling broadly fall into two categories: reference-based and *de novo* alignment (Torkamaneh et al. 2016). If one is available, reads (short sequences) can be mapped to the genome of a related species. Alternatively, if there is no suitable reference genome, as is the case in many ecological studies, similar reads are clustered and aligned to each other (Andrews et al. 2016). In both methods, the quality of the alignment is crucial for downstream SNP calling, as similar sequences may actually be from different areas of the genome (paralogues) (Nielsen et al. 2011). Recent work has demonstrated that SNP calling and some parameter estimates vary substantially across pipelines, with large differences observed between *de novo* and reference-based approaches (Shafer et al. 2017). Thus, more research is needed to fully understand how this key bioinformatic process can impact population genetic analyses, particularly as these can be greatly influenced by incorrectly called genotypes.

Reduced representation techniques usually identify tens of thousands of variants, which are then filtered down to an informative set of loci (Schilling et al. 2014, Shafer et al. 2017). However, filtering strategies vary depending on the specific objectives of each study. For example, genome wide association studies (GWAS) require high coverage of the genome at many loci. This means that low read depths and large amounts of missing data must be tolerated (Glaubitz et al. 2014). Alternatively, for population genetic analyses, it is more appropriate to minimise missing data and retain loci that provide high confidence genotypes across the majority of individuals, even if this means using fewer loci (Schilling et al. 2014). Importantly, the specific goals of the study and the biology of the system should be carefully considered before filtering takes place, to come up with a defensible and informed set of criteria. To date, only a small number of studies have focused on this aspect of bioinformatics (Schilling et al. 2014, Shafer et al. 2017). This is surprising given this step has the potential to either mask or strengthen the biological signals detected by SNP data.

While both SNPs and microsatellites are valuable tools for estimating population genetic parameters, it is also important to recognise the opportunities provided by comparing patterns at genetic markers with different inheritance modes. In fact, mitochondrial (mtDNA) and Y chromosome markers have the potential to provide a different perspective to autosomal SNPs and microsatellites altogether (Allendorf *et al.* 2010; discussed in chapter 2: Shaw *et al.* 2018). These haploid markers do not go through recombination (or have non-recombining regions) and are inherited from one parent only. This means that they can give us a sex-specific perspective on gene flow over longer time scales (Petit et al. 2002, Lawson Handley and Perrin 2007). In mammals, mtDNA markers are maternally inherited, whereas Y chromosome markers are paternally inherited. While mtDNA has been used in population genetics for many years (Sunnucks 2000, Hedrick et al. 2013), NGS is making the addition of Y chromosome markers to ecological research increasingly feasible (Greminger et al. 2010). Taking advantage of these marker types, in combination with autosomal markers, can provide new insights into the demographic processes shaping patterns of genetic structure both within and among populations (Shaw et al. 2018: Chapter 2).

Here, we compare an empirical dataset comprising microsatellite genotypes, mitochondrial DNA sequences and DArTseq^{TM} autosomal and Y chromosome SNP genotypes, across a common set of individuals. We also compare SNPs called using both the proprietary DArT *de novo* approach (Kilian et al. 2012, Cruz et al. 2013), and a reference-based approach (Li et al. 2009). Using a case study of a native Australian rodent, the pale field-rat (*Rattus tunneyi*), from the Kimberley region of Western Australia, we quantify and compare the performance of these marker types for measuring genetic diversity and structure over a fine-scale (tens to thousands of meters) in animal populations. To date, there have been no population genetic studies in pale field-rats and detailed knowledge on demography and dispersal is lacking for this species. This type of information is becoming increasingly important, as pale field-rats are declining across much of their range and demographic information could be vital in

understanding and halting this decline (Cole and Woinarski 2000, Start et al. 2012, Woinarski et al. 2014). Therefore, we quantified:

I. Genetic diversity within populations.
II. Genetic differentiation among populations, separated by 1.5 – 15.5km, using a range of population genetic statistics common in ecological studies of dispersal (G_{ST}, G''_{ST}, D_{est} and Φ_{PT}).
III. Spatial genetic structure among individuals using multilocus spatial autocorrelation, to measure fine-scale genetic structure within populations.

Previous work has highlighted how both biparentally and uniparentally inherited markers can be used to reveal patterns of sex-biased dispersal (Banks & Peakall 2012; Shaw *et al.* 2018: Chapter 2). While this research focuses on fine-scale genetic analyses, these patterns are also likely to be detected at the population level (also discussed in Chapter 2). For this reason, we performed both individual- and population-level analyses separately for females and males to test for signals of sex-biased dispersal. By comparing these metrics among the different marker types and filtering strategies, we explored how they differ in terms of their ability to detect biological signals and sex-specific patterns, and how bioinformatic decisions impact the biological conclusions drawn from fine-scale population genetic analyses.

Methods

Study location and study species

We conducted our study at the Mornington Wildlife Sanctuary (17.55°S, 126.17°E), in the central Kimberley region of Western Australia. Mornington has a monsoonal climate with an average annual rainfall of 750 mm (Bureau of Meteorology). The study area is dominated by open savanna woodlands, made up of tussock and hummock grasses with a sparse eucalypt overstorey. Mornington has been managed for conservation by the Australian Wildlife Conservancy (AWC) since 2004.

The pale field-rat (*Rattus tunneyi*) is a native Australian rodent, and one of a suite of small mammals currently declining across northern Australia (Woinarski et al. 2014).

While populations can fluctuate with seasonal conditions, this species has less irruptive ability than other *Rattus* species (Braithwaite and Griffiths 1996, Start et al. 2012). Pale field-rats make extensive, shallow burrows in sandy soils covering areas up to approximately $20m^2$ (Braithwaite and Griffiths 1996). Home range size is larger in males than in females, covering a mean of 0.39 ha versus 0.09 ha, respectively (Leahy et al. 2015). A peak breeding period usually occurs between March-April (Taylor and Calaby 2004) and generation length is estimated at 1-2 years (Woinarski et al. 2014).

Sampling and DNA extraction

Pale field-rats were trapped at three sites (RS02, RS03 and RS05) from June – August, 2014. The site layout included 100 steel Sherman Type A traps (30 x 10 x 8cm) arranged in two transect lines approximately 30 – 40m apart, with each transect including 50 traps spaced at 20m intervals. We trapped at each site for four nights. Traps were baited with rolled oats and peanut butter late in the afternoon and cleared before sunrise the following day. Ear tissue was collected from 92 unique individuals (RS02: n= 29, RS03: n= 30 and RS05: n= 33) and stored in 70% ethanol. After determining the individual's sex and taking basic measurements, animals were released at the point of capture. DNA was extracted from tissue samples using a proteinase K digestion, followed by protein 'salting out' and ethanol precipitation (Miller et al. 1988).

Molecular markers

Genetic sex determination

In order to confirm the sex of pale field-rats identified in the field, we amplified the male sex-determining region (SRY), with an X-linked microsatellite locus (DX2) used as a positive control (Peakall et al. 2006). PCR conditions followed Peakall *et al.* (2006).

Microsatellites

We genotyped all pale field-rats across 8 autosomal microsatellite loci, using primers originally developed for other *Rattus* species. We tested 32 primer pairs, 12 of which were originally developed for *Rattus fuscipes* and successfully cross-amplified in *R. tunneyi* by Hinten *et al.* (2007). The remaining 20 primer pairs were established by Peakall *et al.* (2006) for testing in *R. fuscipes*, from a public list of mapped microsatellite markers

in the lab rat. PCR reactions and microsatellite amplification followed the protocols of Peakall *et al.* (2006) and Hinten *et al.* (2007). Microsatellites were run with the LIZ500 standard on an Applied Biosystems 3130xl DNA sequencer and scored using Geneious Pro 6.1.4 (Biomatters). We then screened microsatellites for polymorphism and scoring reliability. Finally, we analysed loci in Micro-checker for evidence of allelic drop-out, null alleles and scoring artefacts, and determined if markers conformed to Hardy Weinberg expectations (Van Oosterhout et al. 2004).

Of the 12 sets of primers successfully cross amplified in *R. tunneyi* by Hinten *et al.* (2007), three showed evidence of null alleles (RfgM8, RfgC3 and RfgN4), one was monomorphic (RfgCT2B) and three could not be reliably scored, even after optimisation with annealing temperatures and use of the HotStar HiFidelity Polymerase Kit (Qiagen) (RfgD14, RfgD7, RfgCTGT1B). Of the 20 microsatellite primer pairs established for testing by Peakall *et al.* (2006), 17 either failed to amplify or could not be reliably scored. Therefore, the final *R. tunneyi* panel was RfgG3, RfgO6, RfgW6, RfgW6, RfgL3, RfgL5, D2Rat118, D8Rat123, D15Rat123 (Peakall et al. 2006, Hinten et al. 2007).

SNP markers

DArTseq sequencing

Approximately 500ng of extracted DNA, at a total volume of 10µL per sample, was sent to Diversity Arrays Technology Pty Ltd. where it underwent a Zymo purification step (Zymo Research, California, USA). DArTseq™ SNP genotyping is a proprietary reduced representation method for library preparation and next generation sequencing (Kilian et al. 2012, Cruz et al. 2013). Complexity reduction is achieved through restriction enzyme digestion, which is optimised for each organism. In our case, the enzyme combination *PstI* and *SphI* were selected. After digestion, adapter ligation and PCR amplification, samples were run in a single lane on an Illumina Hiseq2500, and sequenced at approximately 1.5 million reads/sample (or approximately 1.8 million reads/sample if including technical replication).

DArTseq SNP and genotype calling (de novo)

Sequences were processed (including read assembly, quality control and SNP calling) through DArTseq™ proprietary analytical pipelines. This pipeline includes filtering out poor quality sequences, and stringent selection criteria for the barcode region of each sequence (enabling the reliable assignment of sequences to individuals). Identical sequences are then collapsed and used in a secondary pipeline including a proprietary SNP calling algorithm (DArTsoft14) (Melville et al. 2017). Finally, the sequences containing SNPs were BLASTed against the *Rattus norvegicus* reference genome (Rnor6.0; Gibbs *et al.* 2004), estimated to be between 2.7 – 3.8 million years diverged from *R. tunneyi* (Robins et al. 2014). We removed all sex-linked SNPs from this dataset. This included SNPs identified as sex-linked (discussed in more detail below), as well as any SNPs that aligned to the sex chromosomes (550 SNPs in total). The remaining bi-allelic SNPs (58, 909) were classed as the unfiltered DArTseq dataset (although, they were subjected to initial filtering based on quality).

DArTseq SNP Filtering

We identified three main themes for filtering SNP genotypes, to obtain a set of loci appropriate for fine-scale population genetic analyses. These included data quality, allele frequencies and linkage disequilibrium. Data quality filters focused on missing data, reproducibility and read depth, while frequency filters included minor allele frequency (MAF), excess heterozygosity and deviation from Hardy Weinberg expectations. The linkage disequilibrium (LD) filter was employed to avoid the influence of SNP clusters on downstream analyses (Zheng et al. 2012).

We used a custom R script to filter our DArTseq SNPs based on both common thresholds in the literature and our own thresholds aimed at retaining high confidence genotypes. We used a 10% missing data threshold, 95% reproducibility (calculated using DArT technical replicates) and an average read depth of 10 (for both the reference and SNP) (Monostori et al. 2017, Barilli et al. 2018, Gan et al. 2018, Lal et al. 2018). We removed loci with a minor allele frequency below 5% and assumed populations should conform to Hardy Weinberg expectations, and so removed loci that were not in Hardy Weinberg Equilibrium in more than one population (Schilling et al. 2014, Yong et al. 2015, DiBattista et al. 2017, Raman et al. 2017). The latter was carried out using the R package

HardyWeinberg (Graffelman and Morales-Camarena 2008, Graffelman 2015). We also filtered on excess observed heterozygosity, removing SNPs above a threshold of 0.6 since the maximum possible value for bi-allelic markers is 0.5. Finally, we used the R package SNPRelate to calculate pairwise genotypic correlations within a sliding window of 500,000 base pairs, removing SNPs with a correlation of 0.5 or above (Zheng et al. 2012).

Reference-based SNP and genotype calling

To compare DArTseq™ *de novo* SNP calling to an alternative SNP calling method, we obtained the raw FASTQ files (containing both the sequences and the associated quality scores) from Diversity Arrays Technology Pty Ltd. We then used the *R. norvegicus* genome (Rnor6.0; Gibbs *et al.* 2004) to carry out a referenced-based alignment.

Single end sequence reads from each individual (FASTQ format) were mapped to the *R. norvegicus* genome using BWA Mem (Li et al. 2013) with default parameters and saved as SAM files before merging with 'Samtools merge' to a combined BAM format file. Variants were then called with SAMtools Mpileup using the –E parameter, which recalculates the Base Alignment Quality (BAQ) to remove artificial SNPs induced by indels, and BCFtools v1.8 (Li et al. 2009) consensus caller. SAMtools Mpileup uses a probabilistic approach for variant calling, determining the likelihood of each potential genotype using information stored in BAM files. Above a maximum coverage of 255 reads for a variant site, SAMtools randomly samples 255 reads per SNP to reduce the computation time of genotype likelihoods. This information is then used in BCFtools (default parameters), which outputs variants in VCF (Variant Call Format).

The unfiltered VCF file generated above contained 670,140 potential variants and was initially visualised and filtered in R (R Core Team 2017), using the vcfR package (Knaus and Grünwald 2017). We attempted to keep the reference-based SNP dataset as similar as possible to our DArTseq dataset. Therefore, we removed indels and only kept bi-allelic SNPs (removing 99,760 SNPs). We also removed any SNPs that mapped to the sex chromosomes, as well as any unmapped SNPs. We then used the vcfR package to visualise the quality metrics associated with the remaining 556,266 SNPs and determine subsequent filtering thresholds.

SNP genotypes were called in ANGSD (Korneliussen et al. 2014), using genotype likelihoods from the final VCF generated above. Genotype likelihoods are particularly useful for low to medium depth NGS data, as they are determined using probabilistic algorithms that incorporate the error introduced during base calling, alignment and assembly (Nielsen et al. 2011). We generated ANGSD genotypes from these genotype likelihoods, using a uniform prior. We called genotypes using two different posterior probability cutoffs (0.95 and 0.8, with samples below this threshold listed as missing data), in order to determine the impact of using lower confidence genotypes in subsequent analyses. The SNP panels called using these different posterior probability cutoffs are hereafter referred to as the high confidence (0.95) and low confidence (0.8) SNP datasets. SNPs were filtered for quality control in ANGSD based on P-value (≤1*e*-6), mapping quality (≥59.5) and base quality (≥20). The SNPs that passed these initial quality control thresholds (141,573) were classified as the unfiltered reference-based SNP dataset (although much quality-based filtering occurs before this point).

Reference-based SNP Filtering

Reference-based SNPs were filtered following the DArTseq criteria specified above where possible. However, due to differences in our variant calling methods, we were unable to filter on mean depth specifically. We were also unable to filter on reproducibility, as technical replicates that are run with DArTseq were not available for this method. This meant that direct replication of the DArTseq method was not possible. Due to large amounts of missing data, we had to use a more relaxed missing data threshold of 25% for the high confidence dataset and 20% for the low confidence dataset. However, all frequency and LD filters followed the thresholds listed above.

mtDNA and Y chromosome markers

Three mitochondrial regions were sequenced, including COI (using the primers BatL5310 and R6036R), cytochrome *b* (using the primers RGlu2L and RCb9H) and the D-loop (using the primers EGL4L and RJ3R). Primers and PCR conditions were as described in Robins *et al.* (2007) and mtDNA sequencing was conducted using an Applied Biosystems 3130xl DNA sequencer. The raw sequences were edited and aligned in Geneious Pro 6.1.4 (Biomatters) and the three sequences were concatenated to distinguish mtDNA

haplotypes, with the resulting sequence 477 base pairs in length (including 79 polymorphic sites).

We confirmed potential Y-linked SNPs from the DArTseq dataset (identified through BLAST) by calculating heterozygosity and missing data across all individuals with a known sex (consistent across the field ID, SRY and X-linked microsatellite). SNPs were identified as Y-linked if all females showed missing data and all males were homozygotes (allowing for 10% missing data across male samples). The 46 confirmed Y-linked SNPs were then concatenated for haplotype analysis.

We used the software package POPART version 1.7 (http://popart.otago.ac.nz) to construct haplotype networks for both mtDNA and Y chromosome haplotype data, using a median joining network (Bandelt et al. 1999, Leigh and Bryant 2015). This enabled us to visualise haplotype diversity and classify identical sequences (that may differ due to missing data) as the same haplotype for subsequent analyses (Appendix S1).

Statistical analyses

Diversity within populations

Comparisons across markers

We compared genetic diversity among the three populations, for all molecular marker types using the software package GenAlEx 6.5 (Peakall and Smouse 2006, 2012). At autosomal markers (microsatellites and SNPs), we measured the number of alleles (microsatellite loci only), observed heterozygosity (H_o), expected heterozygosity (H_e) and the inbreeding coefficient (F). For mtDNA and Y chromosome markers, the number of unique haplotypes and haplotype diversity was determined.

Comparisons among filters

For both DArTseq and reference-based SNPs, we tallied the number of SNPs retained under each of the three filtering criteria, and for the total filtered datasets. In order to get a broad overview of patterns of heterozygosity within and among populations (relative to Hardy-Weinberg expectations) and to estimate inbreeding, we calculated F_{IS}

(without bias corrections; Nei 1977) across all DArTseq and reference-based (high confidence and low confidence) SNPs. We also compared F_{IS} distributions for SNPs filtered according to the three different filtering criteria (quality, frequency and LD), as well as for the unfiltered and filtered datasets.

Differentiation among populations

Comparisons across markers

We compared patterns of population genetic structure between females and males across our autosomal molecular markers (microsatellites and SNPs) using a number of common population genetic statistics. G_{ST} (Nei 1987, Nei and Chesser 1983; Meirmans & Hedrick 2011), G''_{ST} (Hedrick 2005, Meirmans and Hedrick 2011) and D_{est} (Jost 2008) were calculated in the GenAlEx 6.5 (Peakall and Smouse 2006, 2012). To determine whether population genetic structure was significantly different from zero, and if it differed significantly between the sexes, 95% confidence intervals were calculated using 1000 bootstrap samples over loci. Finally, we used the AMOVA framework to calculate Φ_{PT} (Peakall et al. 1995, Maguire et al. 2002), which is based on an estimation of variance components from genetic distance data in GenAlEx 6.5 (Peakall and Smouse 2006, 2012). This was performed separately for females and males across all marker types, for both the total differentiation and for pairwise population comparisons, testing for statistical significance using 1000 permutations.

Comparisons among filters

To visualise the impact of SNP filtering on population-level structure, we calculated F_{ST} (without bias corrections; interchangeable with Nei's G_{ST}, 1977) between the DArTseq and reference-based SNPs. We compared the distributions of the three independently filtered datasets (quality, frequency and LD), along with the unfiltered and filtered datasets.

Individual-level genetic structure

Correlation between autosomal datasets

To explore the correlation between autosomal datasets, we performed a Mantel test of matrix correspondence (Smouse et al. 1986, Smouse and Long 1992) in GenAlEx 6.5 (Peakall and Smouse 2006, 2012) using individual genetic distance matrices. Individual genetic distances were calculated in R, using the package PopGenReport (Adamack and Gruber 2014), following the method of Smouse & Peakall (1999). Statistical significance was tested using 1000 random permutations.

Genetic Spatial Autocorrelation

We investigated fine-scale spatial genetic structure in pale field-rats by exploring the relationship between genetic and geographical distance, using multilocus spatial autocorrelation analysis in GenAlEx 6.5 (Peakall and Smouse 2006, 2012). This distance-based method allows any data type to be used (multilocus allelic genotypes, bi-allelic SNPs or haplotypes, explored in Chapter 2: Shaw *et al.* 2018), estimating the autocorrelation coefficient, *r*, for individuals over specified distance classes (Smouse and Peakall 1999, Peakall et al. 2003, Double et al. 2005, Banks and Peakall 2012, Blyton et al. 2015). High *r* values represent positive spatial autocorrelation of genotypes (spatial clustering of genetically-similar individuals), with 95% confidence intervals used to detect statistical significance over 1000 bootstraps. Using known home range size to inform our analysis, we used the 'multiple population' approach to estimate *rc* (the combined *r* over populations) for females and males separately across five distance classes of 100 metres each (500 metres in total). We compared these results across all marker types.

Results

Diversity within populations: comparisons across markers

Microsatellites

The number of alleles detected within populations at the eight microsatellite loci ranged from 7 (RfgW6) to 21 (RfgG3) (Appendix S2). Across the three pale field-rat populations,

the mean number of alleles (±SE) over all loci was 13.46 ±0.91 (Table 1). An average observed heterozygosity of 0.86 ±0.020 compared to an average expected heterozygosity of 0.87 ±0.01 resulted in an inbreeding coefficient of 0.01 ±0.02 (Table 1).

DArTseq and reference-based SNPs

The filtered DArTseq dataset displayed a mean observed heterozygosity of 0.25 ±0.001, a mean expected heterozygosity of 0.28 ±0.001 and a positive inbreeding coefficient of 0.10 ±0.002, across all SNP loci and populations (Table 1). Lower levels of heterozygosity were found for the filtered high confidence and low confidence reference-based SNP datasets, with a mean observed heterozygosity of 0.19 ±0.003 and 0.14 ±0.001, a mean expected heterozygosity of 0.24 ±0.003 and 0.21 ±0.002, and a positive mean inbreeding coefficient of 0.18 ±0.006 and 0.26 ±0.004, respectively (Table 1).

mtDNA and Y chromosome haplotypes

The mean number of mtDNA haplotypes detected over all populations (for both females and males) was 5.33 ±0.88 and the mean haplotype diversity was 0.65 ±0.05 (Table 1). The mean number of unique Y chromosome haplotypes detected was lower than at the mtDNA (4.67 ±0.33), although the mean haplotype diversity was higher (0.71 ±0.039; Table 1).

Diversity within populations: comparisons among filters

DArTseq SNPs

The DArTseq SNP dataset was filtered down from 50,615 to 3,763 SNPs when all filtering criteria were applied. When applied independently to the unfiltered dataset, the quality and frequency filters removed the greatest number of SNPs from the dataset (71% and 72%, respectively; Figure 1). SNPs with lower call rates tended to have lower observed heterozygosity relative to expected heterozygosity, resulting in these SNPs deviating significantly from Hardy Weinberg expectations (Figure 2). When applying the quality filter, SNPs were predominantly removed for exceeding the stringent missing data threshold (>10%), whereas the majority of SNPs removed using the frequency filter had minor allele frequencies below 5% (call rate and minor allele frequency distributions are

shown in Figure 3a). The linkage disequilibrium filter removed 33% of SNPs from the unfiltered dataset. However, it is important to note that while many of these SNPs were removed due to possible linkage, this filter also removes unmapped SNPs from the dataset (Figure 1).

Filtering of DArTseq SNPs resulted in considerable differences in the distribution of F_{IS} values across loci (Figure 3b). Overall, mean F_{IS} decreased from 0.423 ±0.001 in the unfiltered dataset, to 0.109 ±0.002 in the final filtered dataset. Relative to the unfiltered dataset, filtering by frequency, LD and quality reduced mean F_{IS} (0.168 ±0.001, 0.357 ±0.002 and 0.173 ±0.002, respectively; Figure 3b). This reduction in F_{IS} was more dramatic for the quality and frequency filters than the LD filter, revealing that missing data and the minor allele frequency have a large impact on observed heterozygosity (relative to the expected).

Reference-based SNPs

Reference-based SNP datasets were filtered down from 143,046 to 645 SNPs (high confidence dataset) and from 180,244 to 1,794 (low confidence dataset). The greatest number of SNPs were removed using the quality filter (high confidence dataset= 98%, low confidence dataset= 91%; Figure 1). The frequency filter removed 80% of SNPs from the high confidence dataset compared to 90% in the low confidence dataset. Finally, the LD filter removed 84% and 79% of SNPs in the high and low confidence datasets, respectively (Figure 1). Compared to the DArTseq SNP dataset, the reference-based SNPs displayed a lower call rate (particularly in the high confidence dataset; Figure 2, Figure 3a). However, minor allele frequency distributions were similar between all SNP datasets (Figure 3a).

The distribution of F_{IS} values for the reference-based SNPs varied under the different filtering criteria (Figure 3b). Across the high confidence SNPs, mean F_{IS} for the unfiltered dataset was 0.224 ±0.001, compared to 0.213 ±0.006 for the final filtered dataset. Relative to the unfiltered high confidence dataset, filtering by quality increased mean F_{IS} (0.255±0.001), whereas frequency and LD filters reduced mean F_{IS} (0.198 ±0.001 and 0.225 ±0.002, respectively). Alternatively, mean F_{IS} values across the low confidence

dataset were higher than both the high confidence reference-based dataset and the DArTseq dataset (Figure 3b). This was true for the unfiltered (0.589 ±0.001), quality filtered (0.459 ±0.002), frequency filtered (0.278 ±0.002), LD filtered (0.509 ±0.002) and the final filtered datasets (0.322 ±0.003).

Differentiation among populations: comparisons across markers

Low but significant population genetic structure was detected for both females and males across all autosomal markers and differentiation metrics. Furthermore, females consistently showed higher levels of population genetic structure than males. However, this difference was not significant across all datasets and the magnitude differed across marker types. These results are summarised in Figure 4.

Microsatellites

At microsatellite markers, significant positive structure was detected across all metrics, with lower levels of population genetic structure found in males than females (although 95% bootstrap confidence intervals overlapped; Figure 4). Standardised G''_{ST} [0,1] and Jost's D_{est} were an order of magnitude higher than G_{ST} (G''_{ST}: females= 0.171, males= 0.096; Jost's D_{est}: females= 0.153, males= 0.087; G_{ST}: females= 0.014, males= 0.007), as expected since G_{ST} (and F_{ST}) is generally underestimated in multi-allelic markers. Confidence intervals around the means increased when using the corrected metrics.

DArTseq SNPs

In contrast to microsatellite markers, the difference in population genetic structure between females and males was significant as measured by all of the differentiation metrics across the DArTseq SNPs (female and male 95% bootstrap confidence intervals did not overlap; Figure 4). The level of population genetic structure measured by G''_{ST} doubled (females= 0.031, males= 0.023) and the confidence intervals about this metric increased, as compared to G_{ST} (females= 0.015, males= 0.011). However, this increase was not as dramatic as for microsatellite markers, given these markers are bi-allelic. The lowest level of population genetic structure was detected using Jost's D_{est} (females= 0.009, males= 0.007).

Reference-based SNPs

While population genetic structure measured at reference-based SNPs was similar to that found for DArTseq SNPs, 95% confidence intervals increased, resulting in overlapping confidence intervals between the sexes (Figure 4). The level of population genetic structure measured across the high confidence reference-based SNPs (G_{ST}: females= 0.013, males= 0.008; G''_{ST}: females= 0.026, males= 0.017; Jost's D_{est}: females= 0.006, males=0.004) was lower than that found across the low confidence reference-based SNPs (G_{ST}: females= 0.015, males= 0.011; G''_{ST}: females= 0.028, males= 0.021; Jost's D_{est}: females= 0.006, males= 0.005).

Φ_{PT} comparisons across all markers

Total Φ_{PT} patterns across markers again revealed low, but significant, levels of population genetic structure (Figure 5a). Females consistently showed higher levels of population genetic structure than males across all autosomal markers (females: 0.034 – 0.042; males: 0.018 – 0.027). Females also showed significantly higher structure than males at mtDNA markers (females: 0.076 vs. males: 0.003) and when comparing female mtDNA to male Y chromosome markers (females: 0.076 vs. males: 0.064; Figure 5a). In general, this was also true for pairwise population comparisons of Φ_{PT}, with females showing greater levels of structure than males across the different marker types (Figure 5b). However, these patterns began to break down for haplotype markers, most likely due to the small sample measured at just one locus (haplotype). Population differentiation was greatest for pairwise comparisons to RS03, which was also the most geographically distant population. Surprisingly, while comparatively lower levels of genetic structure were found when comparing RS02 and RS05 (compared to that found for RS03), these populations still showed significant population differentiation at the SNP markers for both sexes (apart from the high confidence reference-based SNPs) despite these sites only being 1.5km apart.

Differentiation among populations: comparisons among filters

DArTseq SNPs

The impact of SNP Filtering on the distribution of F_{ST} values over the DArTseq dataset was not as pronounced as it was for F_{IS}, with a mean F_{ST} of the unfiltered dataset of 0.04

±2.1E-4, compared to a mean of 0.02 ±4.0E-4 for the final filtered dataset (Figure 3b). Furthermore, F_{ST} values across SNPs were less variable, and did not appear to be strongly related to call rate (Figure 2; Figure 3b). Mean F_{ST} ranged from 0.025 to 0.037 over the different filtering criteria (Figure 3b). While the differences were much smaller than those found for F_{IS}, these patterns were consistent, with all filters reducing F_{ST}, although the quality filter had the greatest impact on mean F_{ST} (quality filter = 0.02 ±2.0E-4; frequency filter = 0.03 ±2.4E-4; LD filter= 0.04 ±3.0E-4; Figure 3b).

Reference-based SNPs

The reference-based SNPs showed similar distributions of F_{ST} to the DArTseq SNP dataset (Figure 3b). F_{ST} was similar for both the high and low confidence reference-based SNP datasets, with a mean of 0.22 ±8.0E-4 (high confidence) compared to 0.17 ±6.2E-4 (low confidence) for the unfiltered datasets, and 0.03 ±1.0E-3 (high confidence) compared to 0.03 ±6.3E-4 (low confidence), for the final filtered dataset (Figure 3b). The quality filter reduced mean F_{ST} to a similar level as the final filtered dataset (high confidence dataset= 0.03 ±5.1E-4; low confidence dataset= 0.03 ±2.0E-4). The frequency and LD filters also reduced mean F_{ST} compared to the unfiltered dataset, though to a lesser extent than the quality filter (high confidence dataset: frequency filter= 0.05 ±3.3E-4, LD filter= 0.06 ±7.6E-4; low confidence dataset: frequency filter= 0.05 ±3.9E-4, LD filter= 0.05 ±3.5E-4).

Individual-level genetic structure: correlation between autosomal datasets

A significant positive correlation was found between all autosomal datasets (Figure 6). This correlation was highest for the DArTseq and low confidence reference-based SNP datasets (Rxy= 0.53). However, all SNP datasets showed similar levels of correlation (DArTseq vs. high confidence reference-based SNPs: Rxy= 0.41; high confidence vs. low confidence reference-based SNPs: Rxy= 0.46; Figure 6). Microsatellite markers were most strongly correlated with DArTseq SNPs (Rxy= 0.28), followed by the low confidence (Rxy= 0.19) and the high confidence reference-based SNPs (Rxy= 0.16; Figure 6).

All markers showed clear correlations when individual comparisons of genetic distances were within sites (<1000m), and thus were all able to detect high levels of relatedness (indicated by low pairwise genetic distance). However, once comparisons

were between pairwise individuals captured in different populations, genetic distances calculated across the DArTseq SNPs showed greater spread that more closely aligned with the geographic distance than any of the other autosomal markers (Figure 6).

Individual-level genetic structure: genetic spatial autocorrelation

While correlograms for all autosomal marker types (summarised in Figure 7) consistently revealed higher levels of fine-scale genetic structure in females than males, female and male confidence intervals overlapped for all marker types. The DArTseq SNPs showed significant positive genetic spatial autocorrelation for both females and males at 100m. However, 95% bootstrap confidence intervals overlapped zero for both the high and low confidence reference-based SNPs at this distance class. While significant positive genetic spatial autocorrelation was detected for females at microsatellite markers, this was not true for males, with confidence intervals overlapping zero. At mtDNA markers, females displayed positive fine-scale genetic structure (though not significant), whereas male structure was close to zero. Conversely, significant positive genetic structure detected at the male Y chromosome was greater than that found for females at the mtDNA, however, 95% confidence intervals were large for both haplotype markers. In all cases, the level of fine-scale structure (*rc*) quickly declined to zero by the second distance class (200m).

Discussion

Common methods for SNP genotyping involve reduced representation library preparation from multiple barcoded individuals followed by NGS. These methods can identify up to hundreds of thousands of likely SNPs, but stringent filtering is required if we are to have the confidence in individual genotypes that is required for fine-scale population genetic analyses. Several key issues arise using these methods, including: (1) Are we looking at a homologous or paralogous locus?; (2) Do we have enough sequence information to accurately call the genotype of a given individual at a SNP locus? (3) Is the SNP (or individual) informative for population genetic analyses? To address these issues, there are a number of different approaches to bioinformatic SNP calling (reference and *de novo*) and filtering that can be applied to NGS datasets, although all of these methods have pros and cons. Importantly however, decisions about bioinformatic pipelines and

filtering should be justifiable, with careful consideration of the biology of the system and the specific goals of the study.

Diversity within populations: comparisons across markers and pipelines

Multi-allelic microsatellite markers commonly show higher levels of heterozygosity than SNPs, as SNPs are bi-allelic and thus can only reach a maximum heterozygosity of 0.5. (Cappa et al. 2016). However, the discrepancy in average heterozygosity between DArTseq and reference-based SNPs was surprising, given all were generated using the same sequence data. This difference highlights the influence that error (introduced through calling SNPs using low read depth data) can have on individual genotypes (and thus observed heterozygosity) in reduced representation SNP calling methods. Observed heterozygosity was lowest for the low confidence reference-based SNPs, suggesting that individual genotypes were likely based on a single occurrence of the SNP or reference allele and thus heterozygotes were undercalled. This improved with the high confidence reference-based SNP dataset, as individual genotypes with low posterior probabilities (due to low quality bases or low individual read depth) were instead included as missing data. Unfortunately, the proprietary nature of DArTseq means that it is not possible to know what the read depth thresholds and posterior probabilities used for genotype calling are for this method. However, the expected and observed heterozygosity were closest for these SNPs, suggesting that heterozygotes were not under called to the same extent in this dataset.

SNP calling methods face a trade off with low to medium depth data (common in reduced representation studies). Either heterozygotes are underrepresented or too many false SNPs are included in the dataset (Nielsen et al. 2012). This is because, with low read depth, there are very few sequences with which to call a heterozygote. For example, if a heterozygous individual has 10 reads at a SNP locus, ideally they should have 5 copies of each allele. However, it is also possible that only one copy of the SNP and nine copies of the reference allele will be present. Thus, bioinformatic pipelines must determine if polymorphisms represent genuine heterozygotes, or sequencing error. There are many bioinformatic pipelines to choose from when using reduced representation methods, each dealing with these issues in different ways (Andrews et al. 2016). This has been of growing concern, as some studies have observed strong effects

of pipeline on genetic summary statistics (Hohenlohe et al. 2010, Shafer et al. 2017). This suggests that biological inferences based on reduced representation data are likely to be somewhat shaped by bioinformatic parameters.

Diversity within populations: comparisons among filtering criteria

Estimates of F_{IS} were strongly influenced by filtering. Relative to the unfiltered datasets, filtering only on the frequency criteria reduced F_{IS}. The majority of loci removed by this filter were below the minor allele threshold. This is important for removing potential sequencing errors, which can then be misidentified as alternate alleles (Glaubitz et al. 2014). Furthermore, Glaubitz et al. (2003) suggested that these low frequency SNPs are unlikely to be informative for fine-scale genetic analyses. However, the removal of SNPs that did not conform to Hardy Weinberg expectations in the majority of populations was likely the main driver in reducing mean F_{IS} (Figure 2).

Filtering on LD reduced estimates of F_{IS} relative to the unfiltered datasets. However, filtering on linkage alone may not retain the most informative SNPs. Nonetheless, including linked markers in population genetic analyses can result in overestimation and overconfidence in results (Jones and Wang 2010), thus F_{IS} estimates decreased once non-independent SNPs were removed from the dataset. For this reason, we suggest applying LD filters last so that important biological signals are maintained in the dataset.

Finally, the quality filter generally returned estimates of F_{IS} with a magnitude much closer to expectations (given the microsatellite results and the final filtered datasets). In this filter, the majority of SNPs were removed due to excess missing data. Observed heterozygosity appeared to be negatively correlated with the percentage of missing data (Figure 2), suggesting that this factor can greatly influence population diversity estimates. The amount of missing data can vary across different methods and bioinformatic pipelines (for example, based on posterior probability cutoffs as in our high and low confidence reference-based SNP datasets). Therefore, it is important for researchers to determine how missing data influences results and thus how much can be tolerated for each specific study.

Differentiation among populations: comparisons across markers and pipelines

Estimates of population genetic structure (G_{ST}, G''_{ST}, D_{est} and Φ_{PT}) varied over the different marker types. In general, microsatellites, DArTseq SNPs and reference-based SNPs revealed low, but significant population structure for both females and males. Higher levels of population structure were detected in females than males. However, this difference was only significant at the DArTseq SNPs. This is likely due to the higher number of SNPs retained after filtering the DArTseq dataset (compared to the reference-based datasets), resulting in tighter confidence intervals about the estimates. The DArTseq results are consistent with previous work that found SNPs can provide greater accuracy and precision for fine-scale population genetic analyses compared to microsatellite markers, particularly in the absence of strong population structure (Larson et al. 2014, Steane et al. 2015).

Low levels of population genetic structure were also found at mtDNA and Y chromosome markers. Uniparentally inherited markers are expected to have half the effective population size of biparentally inherited markers, meaning they typically display larger magnitudes of population genetic structure. Thus, the low magnitude of Φ_{PT} estimates across both uniparentally and biparentally inherited markers suggests that there are high levels of gene flow across the landscape. Chapter 2 (Shaw et al. 2018) demonstrates that mtDNA and Y chromosome markers can reveal sex-specific patterns of gene flow, which are also likely to be detected over the landscape scale. Indeed, here we found significant population genetic structure for females at mtDNA markers, while male mtDNA and Y chromosome structure did not differ from null expectations (no population genetic structure). Thus, the presence of congruent patterns across mtDNA, Y chromosome, microsatellites and DArTseq SNPs and reference-based SNPs provides some evidence for male-biased dispersal.

While some research has concluded that estimates of F_{ST} are fairly robust across different filtering strategies (Shafer et al. 2017), others have found differences in the magnitude of F_{ST} estimates (Schilling et al. 2014). Here, we found that the magnitude of F_{ST} and the sex-specific patterns detected were fairly robust across filtering criteria and bioinformatic pipelines. However, while certainly not considerable, estimates based on the reference-based SNPs showed the lowest levels of population genetic structure of all

markers and did not reveal significant patterns of male-biased dispersal. Similarly, Hohenlohe et al. (2010) found reduced levels of within population diversity and population differentiation in a study of threespine sticklebacks (*Gasterosteus aculeatus*), using reference-based, RAD-seq SNPs. They suggested that this was likely due to conservative SNP calling.

Differentiation among populations: comparisons across metrics

As expected, the magnitude of estimates varied across the range of common population genetic statistics we used to investigate dispersal. This is because these metrics have key differences in the way they estimate population differentiation. While G_{ST} (Nei 1973; Nei & Chesser 1983) is the most widely used statistic for investigating population-level genetic structure, it is highly dependent on the average heterozygosity within populations (HS). This lead to the development of G''_{ST} (Hedrick 2005, Meirmans and Hedrick 2011), which takes into account the maximum possible value G_{ST} can achieve given the observed within population diversity. Alternatively, Jost's D_{est} (2008), is based on allelic differentiation, while the analysis of molecular variance framework (AMOVA) partitions variance components among populations (Meirmans 2006).

As expected, corrections based on HS and measures based on allelic differentiation (G''_{ST}, D_{est}) made a substantial impact to the magnitude of microsatellite results, as these markers displayed high allelic diversity and observed heterozygosity. Furthermore, when using these metrics, male population genetic structure increased so that confidence intervals no longer overlapped zero. Heller & Siegismund (2009) used a meta-analysis to demonstrate that the magnitude of G_{ST} was consistently lower than D_{est}. They also showed that G''_{ST} is more comparable to $D_{est,}$ especially when markers show high HS. Our microsatellite results certainly reflect these patterns. However, the variance is much higher for these estimates, particularly when heterozygosity is high (Meirmans and Hedrick 2011).

Individual spatial analysis

A Mantel test of pairwise individual genetic distances showed significant, positive correlations between all autosomal markers (compared to null expectations). Furthermore, pairwise genetic distances at DArTseq SNPs more closely reflected

geographic relationships than the other markers, suggesting this dataset has higher resolution to detect patterns of relatedness in pale field-rats (though comparisons between individuals that were more than 1000m apart still overlapped).

Multilocus spatial autocorrelation analysis detected significant positive structure over a spatial scale of 100m across DArTseq SNPs and microsatellite markers. Female fine-scale genetic structure was greater than that found for males across all autosomal markers. However, again the DArTseq SNPS showed higher resolution to detect significant patterns at this fine scale, showing significant structure for both males and females. The large confidence intervals and a lack of significant difference in sex-specific structure may reflect a lack of power to detect such patterns. However, mostly consistent patterns across all marker types implies that high variability in fine-scale structure is a true biological result that warrants further investigation.

Conclusions

One ubiquitous downfall of reduced representation sequencing is the resulting low read depth data. Thus bioinformatic pipelines often need to incorporate some kind of probabilistic method for assigning individual genotypes when there may not be adequate data to call these based on actual sequence information. Even commercial companies (such as DArTseq) are likely to face these issues, however, this may be screened behind the 'black box' of proprietary analysis. This is not necessarily a problem (as there are lots of benefits to using this type of data), however it is critical that researchers are aware that the way in which these challenges are dealt with can shape population genetic results.

Here, we show that both bioinformatic pipelines and filtering can have a strong impact on population genetic estimates based on observed heterozygosity. These results add to a growing body of research highlighting the strong impact that bioinformatic processing can have on downstream analyses, particularly diversity estimates such as F_{IS} (Schilling et al. 2014, Shafer et al. 2017). Importantly, we found that population-level and distance-based metrics were fairly robust to the different bioinformatic pipelines and filtering. However, there was a trade-off between conservative SNP calling and a loss of resolution to detect fine-scale patterns (through stringent filtering removing a large

number of SNPs). Unfortunately, there is no one correct way to process and filter NGS reduced representation data. However, it is clear that thoroughly exploring SNP data and understanding how data processing can shape results should be a critical part of any study using this type of data.

Tables and Figures

Table 1. Summary statistics (± standard error) across marker types and pale field-rat populations. Number of samples (*N*), number of alleles (N_A), observed heterozygosity (H_O), expected heterozygosity (H_E), inbreeding coefficient (*F*), number of unique haplotypes (N_H) and haplotype diversity (*HD*) are presented.

Marker	No. Loci	Summary Statistics	RS02	RS03	RS05	Total
		N	29	30	33	92
Microsatellites	8	N_A	14.12 ±1.737	12.62 ±1.603	13.62 ±1.546	13.46 ±0.909
		H_O	0.83 ±0.038	0.85 ±0.039	0.90 ±0.018	0.86 ±0.020
		H_E	0.87 ±0.018	0.86 ±0.018	0.87 ±0.019	0.87 ±0.010
		F	0.05 ±0.029	0.02 ±0.038	-0.03 ±0.019	0.01 ±0.018
DArTseq SNPs (Bi-allelic)	3763	H_O	0.25 ±0.002	0.25 ±0.002	0.25 ±0.002	0.25 ±0.001
		H_E	0.28 ±0.002	0.28 ±0.002	0.28 ±0.002	0.28 ±0.001
		F	0.10 ±0.004	0.10 ±0.004	0.10 ±0.004	0.10 ±0.002
Ref-Based SNPs 0.95 Cutoff (Bi-allelic)	645	H_O	0.19 ±0.005	0.19 ±0.005	0.19 ±0.005	0.19 ±0.003
		H_E	0.25 ±0.005	0.24 ±0.005	0.24 ±0.006	0.24 ±0.003
		F	0.19 ±0.011	0.18 ±0.011	0.16 ±0.010	0.18 ±0.006
Ref-Based SNPs 0.8 Cutoff (Bi-allelic)	1794	H_O	0.14 ±0.002	0.14 ±0.002	0.14 ±0.002	0.14 ±0.003
		H_E	0.21 ±0.003	0.21 ±0.003	0.21 ±0.003	0.21 ±0.003
		F	0.26 ±0.007	0.26 ±0.007	0.27 ±0.007	0.26 ±0.006
mtDNA	1	N_H	7	5	4	5.33 ±0.882
		HD	0.75	0.64	0.57	0.65 ±0.052
Y chromosome (males)	1	N_H	5	4	5	4.67 ±0.333
		HD	0.71	0.64	0.78	0.71 ±0.039

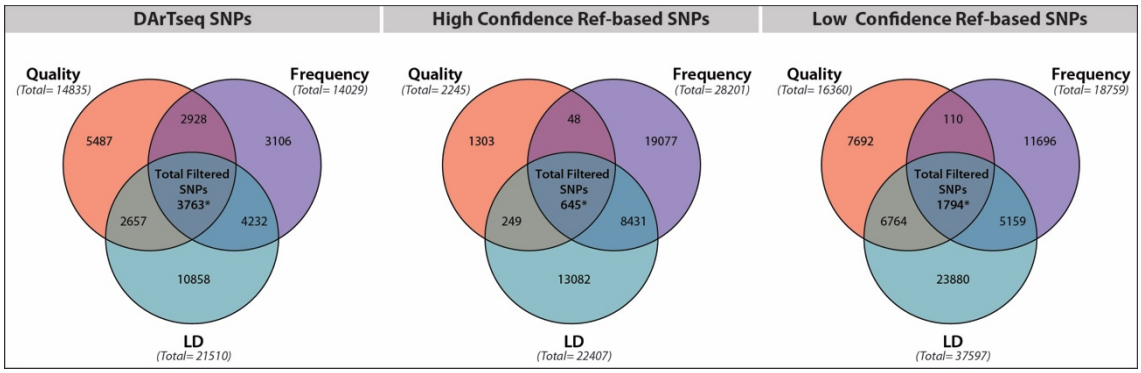


Figure 1. Venn diagrams (Chen 2018) showing the number of SNPs retained after filtering across three criteria (quality, frequency and linkage disequilibrium), for DArTseq and reference-based SNP datasets. The overlapping areas represent the number of common SNPs retained between the different criteria, and for the total filtered dataset*.

**Filters were applied independently to the unfiltered dataset. To obtain our total filtered dataset, we applied the filters in this order: quality, frequency, LD. Since the LD filter will remove different SNPs depending on the input dataset, the number of SNPs is not a function of the overlap of all three independently applied filters (hence there is a slight discrepancy in the number of SNPs in the total filtered dataset).*

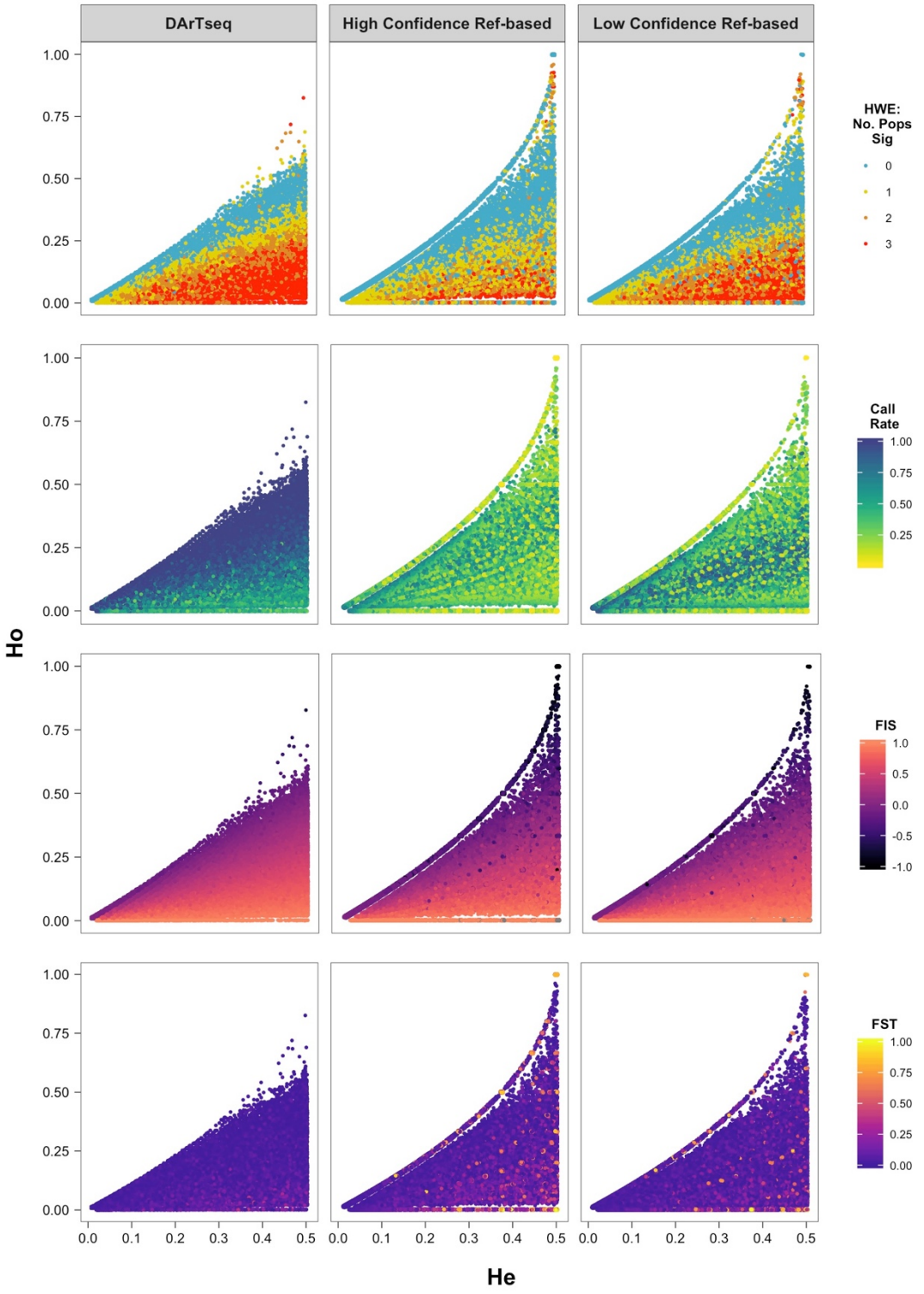


Figure 2. Observed versus expected heterozygosity across the three SNP datasets. SNPs are coloured according to whether they conform to Hardy Weinberg expectations (i.e. the number of populations they are significantly out of Hardy Weinberg Equilibrium in), call rate, F_{IS} and F_{ST}.

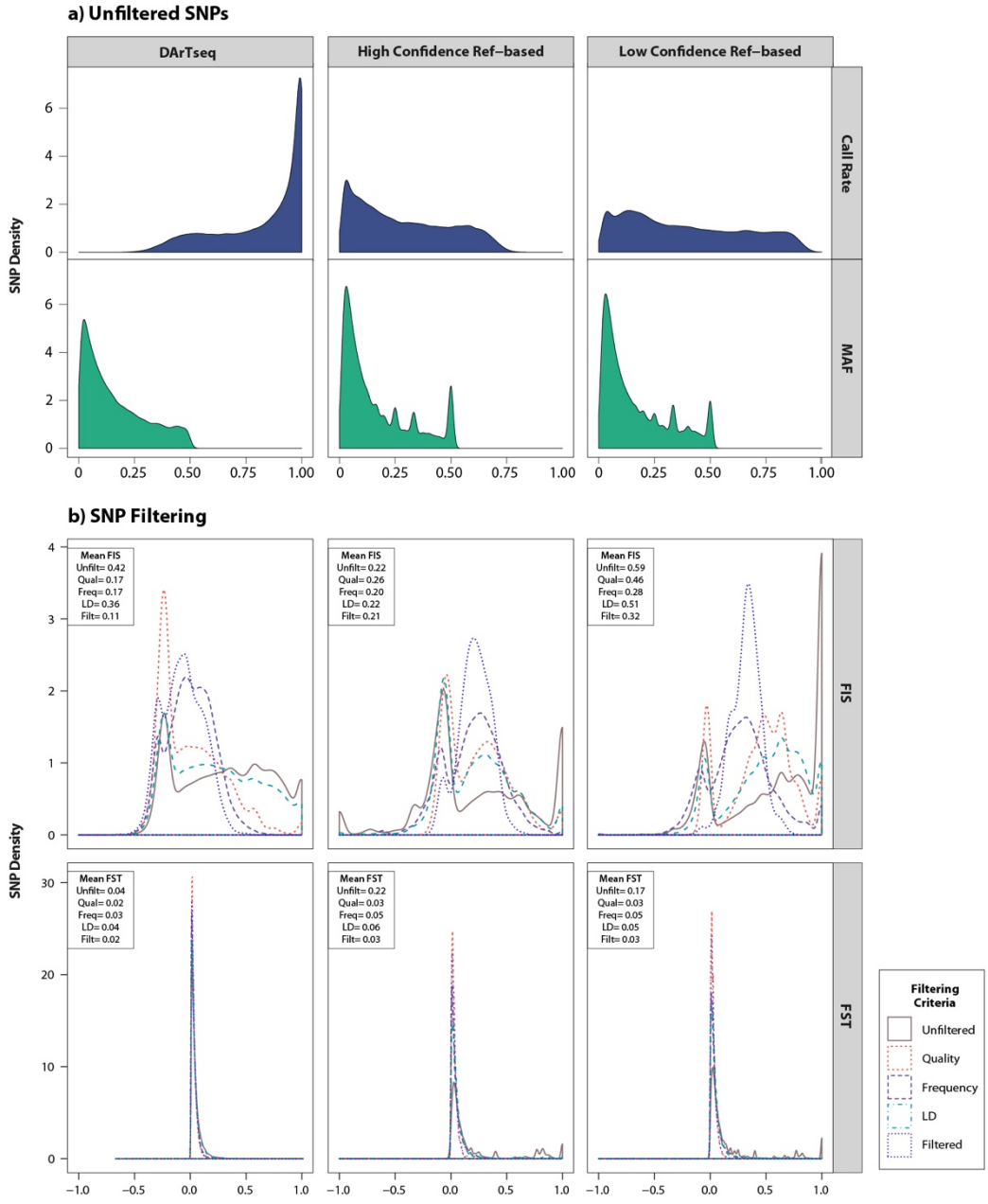


Figure 3. Kernel density estimates (Wickham 2009) for DArTseq and reference-based SNPs showing a) call rate (proportion of individual genotypes called at a SNP locus) and minor allele frequency (MAF) distributions for unfiltered SNP datasets; b) F_{IS} and F_{ST} distributions (without bias corrections; interchangeable with Nei's G_{IS} and G_{ST}, 1977) across the three filtering criteria (quality, frequency and linkage disequilibrium), the unfiltered datasets and the final filtered datasets.

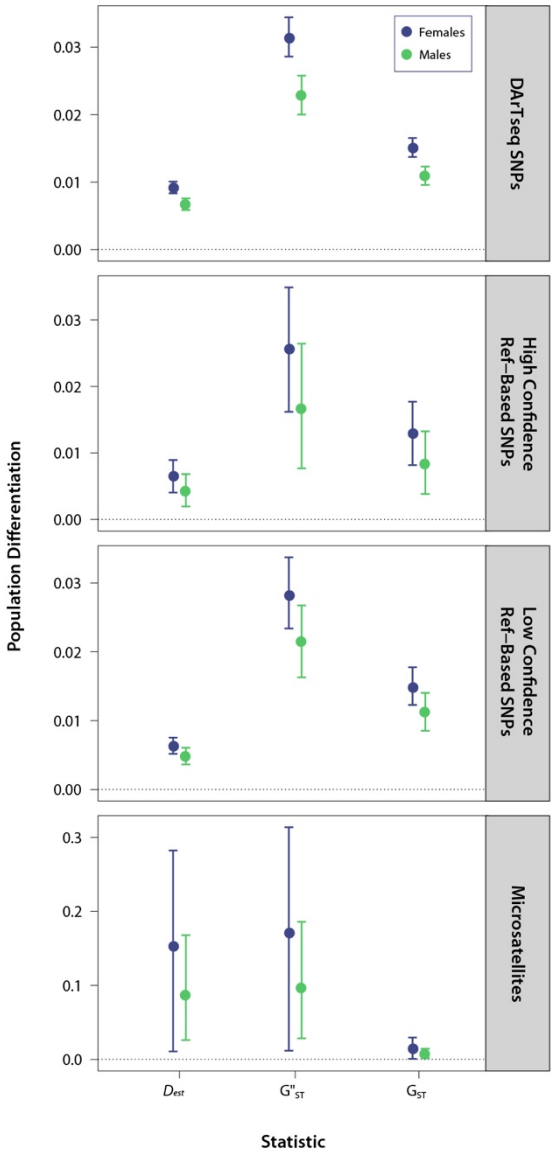


Figure 4. Estimates of population differentiation across pale-field rat populations for the autosomal molecular markers, measured using a number of common metrics. Estimates are bounded by 95% bootstrap confidence intervals, generated using 1000 bootstrap samples over loci.

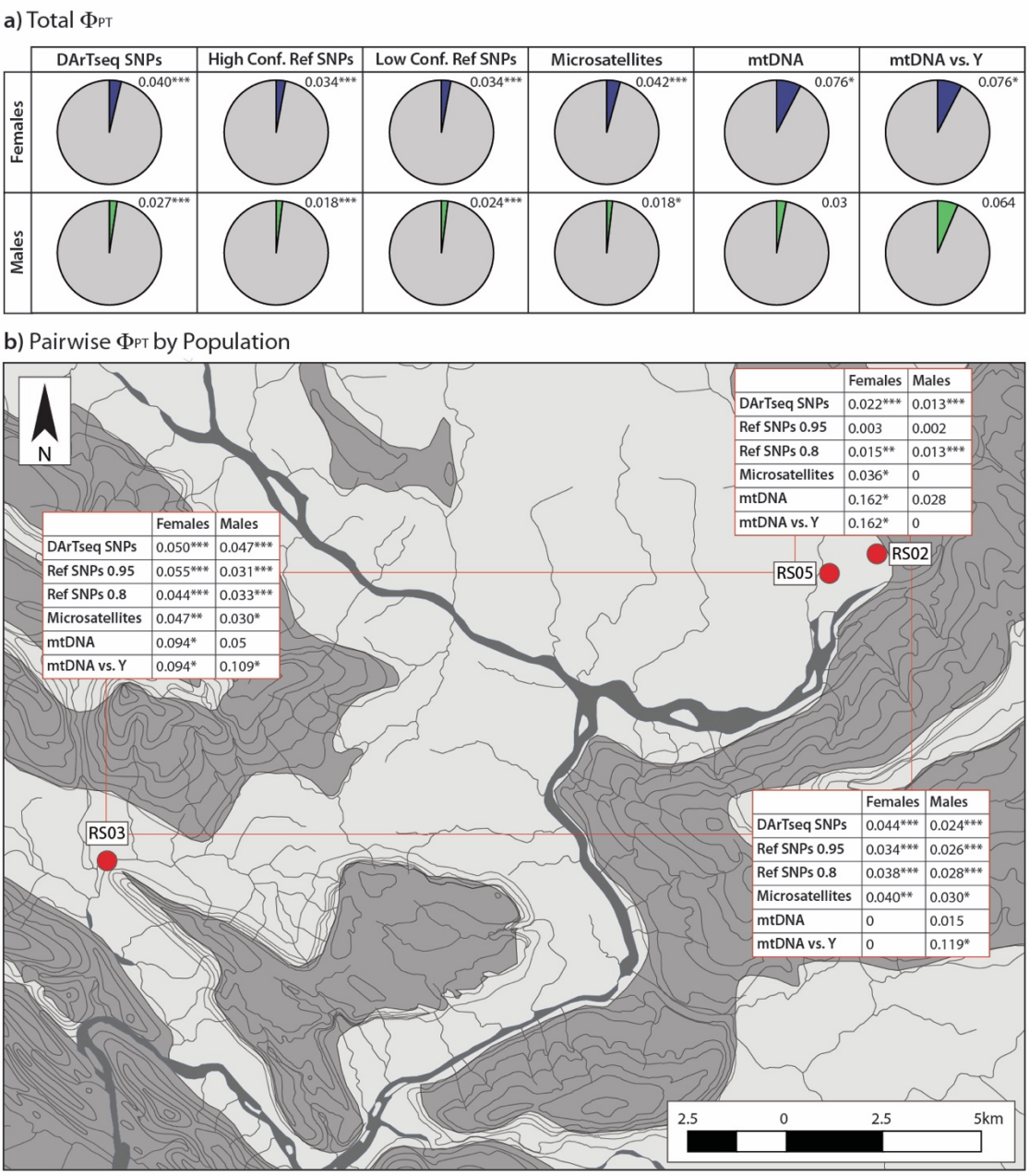


Figure 5. Population genetic differentiation as measured by Φ_{PT} across all molecular markers for a) total population differentiation. Pie charts represent among population genetic variation (blue and green) and among individual genetic variation (grey). b) Pairwise population Φ_{PT} across a map of the Mornington Wildlife Sanctuary, including low-land savanna (light grey), sandstone bluffs and ranges (grey) and creeks and rivers (dark grey). Statistical significance (*<0.05, **<0.01, ***<0.001) was determined with 1000 random permutations.

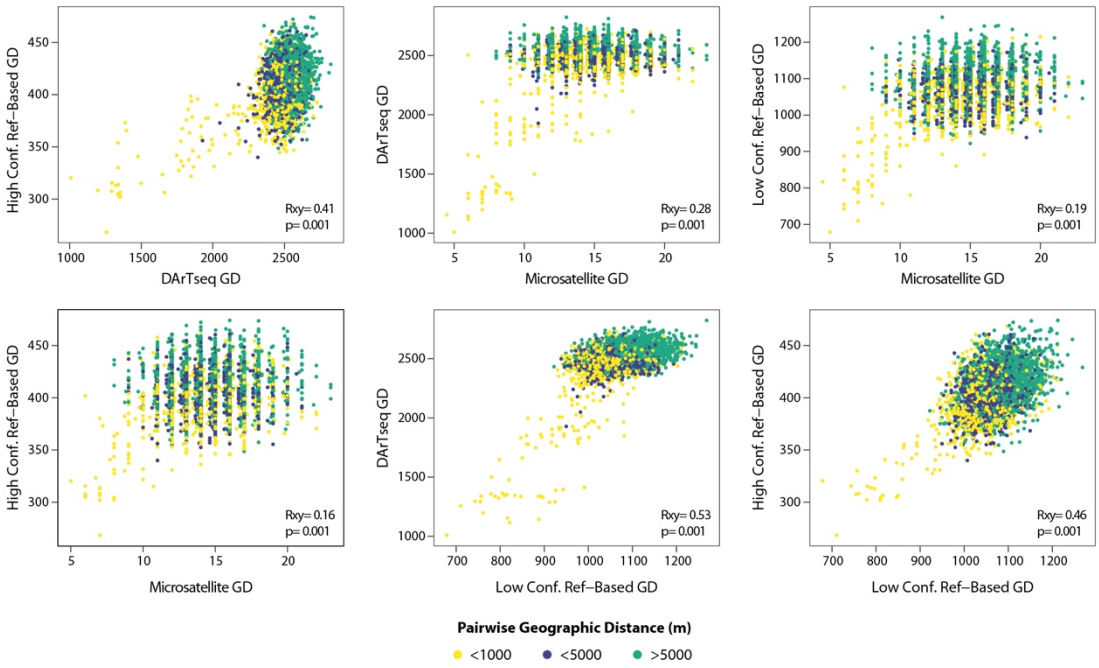


Figure 6. Mantel tests for matrix correspondence of pairwise individual genetic distances calculated across autosomal markers (microsatellites, DArTseq SNPs, high confidence reference-based SNPs and low confidence reference-based SNPs). Statistical significance was determined with 1000 random permutations. Colours represent the geographic distance between the individuals compared. Individuals less than 1000m apart are from the same population.

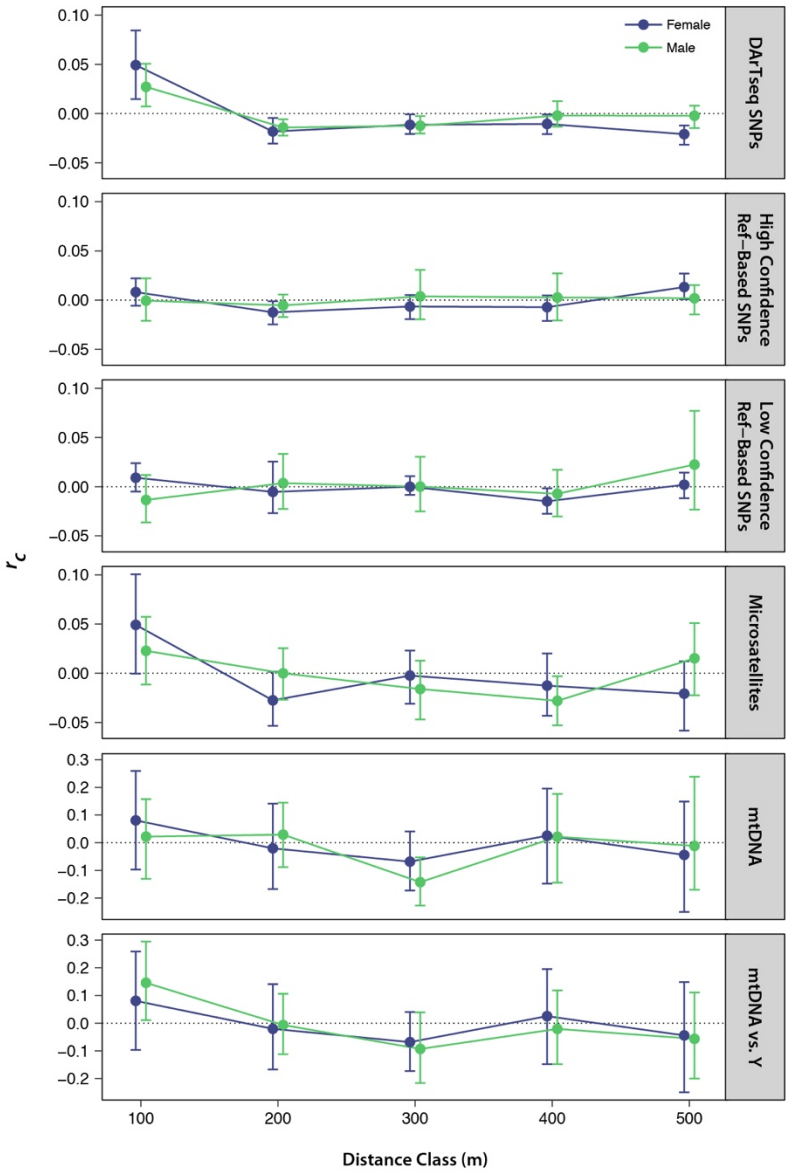


Figure 7. Correlograms displaying genetic spatial autocorrelation results across all molecular markers, for five distance classes (100m). Estimates are bounded by 95% bootstrap confidence intervals.

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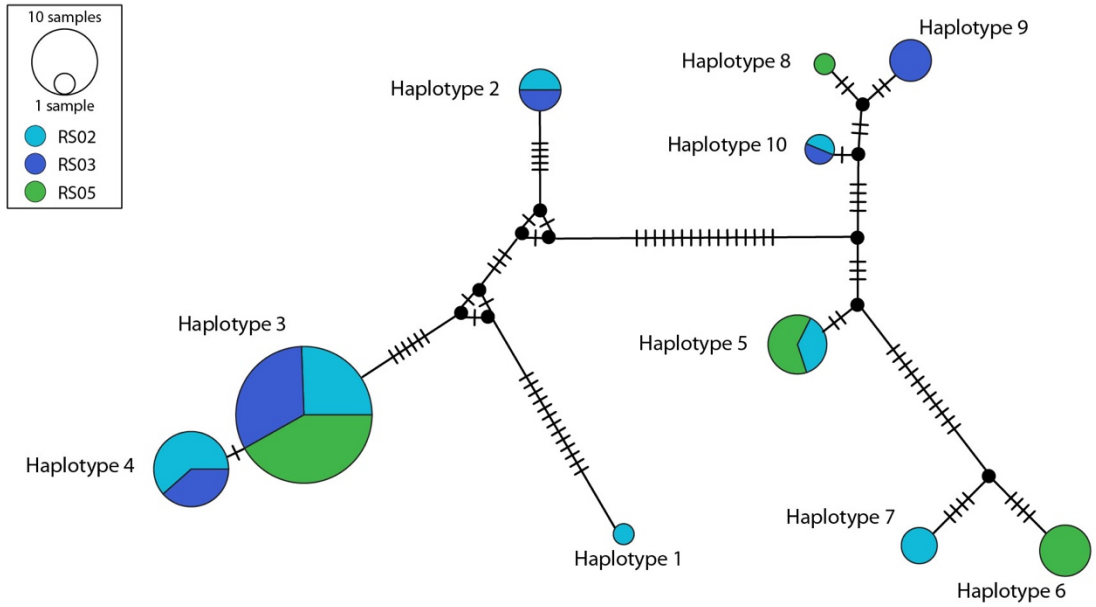
a)



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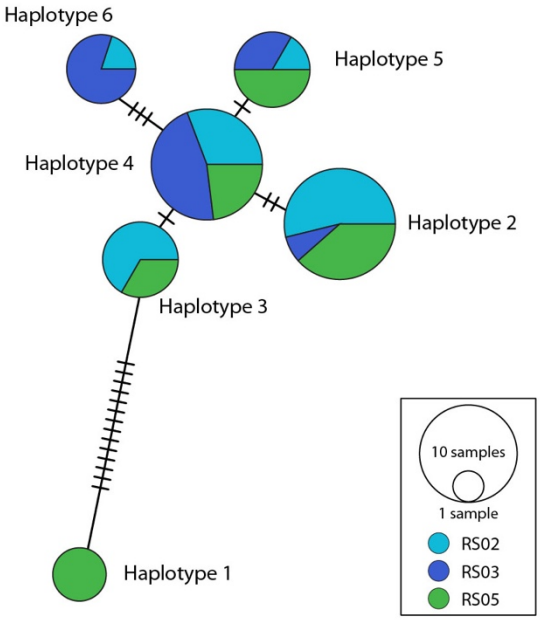

Appendix S1. Haplotype networks for a) mtDNA haplotypes for both females and males and b) Y chromosome haplotypes for males only. Dashes represent the number of mutational steps between haplotypes.

Appendix S2. Summary statistics across microsatellite loci for the three pale field-rat populations. Number of alleles (N_A), observed heterozygosity (H_O), expected heterozygosity (H_E) and inbreeding coefficient (F) are presented.

Population	Locus	N_A	H_O	H_E	F
RS02	D2RAT118	17.000	0.759	0.825	0.080
	D8Rat123	14.000	0.931	0.913	-0.020
	G3	23.000	0.963	0.938	-0.027
	D15RAT123	15.000	0.862	0.894	0.035
	RfgO6	11.000	0.759	0.877	0.135
	RfgW6	7.000	0.731	0.781	0.064
	RfgL3	16.000	0.966	0.905	-0.067
	RfgL5	10.000	0.708	0.859	0.175
RS03	D2RAT118	16.000	0.897	0.900	0.004
	D8Rat123	13.000	0.893	0.881	-0.014
	G3	21.000	0.967	0.928	-0.042
	D15RAT123	12.000	0.867	0.856	-0.012
	RfgO6	10.000	0.655	0.860	0.238
	RfgW6	7.000	0.700	0.782	0.105
	RfgL3	14.000	0.933	0.890	-0.049
	RfgL5	8.000	0.867	0.786	-0.102
RS05	D2RAT118	14.000	0.818	0.875	0.065
	D8Rat123	16.000	1.000	0.917	-0.091
	G3	21.000	0.939	0.923	-0.018
	D15RAT123	16.000	0.939	0.910	-0.032
	RfgO6	9.000	0.879	0.828	-0.061
	RfgW6	8.000	0.839	0.775	-0.082
	RfgL3	15.000	0.939	0.900	-0.043
	RfgL5	10.000	0.818	0.844	0.030
Total	D2RAT118	15.667	0.824	0.867	0.050
	D8Rat123	14.333	0.941	0.903	-0.041
	G3	21.667	0.956	0.929	-0.029
	D15RAT123	14.333	0.889	0.887	-0.003
	RfgO6	10.000	0.764	0.855	0.104
	RfgW6	7.333	0.756	0.780	0.029
	RfgL3	15.000	0.946	0.898	-0.053
	RfgL5	9.333	0.798	0.830	0.034

Chapter 4

Habitat preferences, fire response and recovery in an Australian native rodent

Abstract

Fire is a defining characteristic of savanna landscapes. Despite this, we currently lack a detailed understanding of how animal populations recover after fire. In Australia's northern savannas, small mammal populations are collapsing. Evidence suggests the interaction between altered fire regimes and other key threats is responsible for these declines. Therefore, studies elucidating the post-fire recovery process can be used to develop more effective management strategies that support conservation of vulnerable species.

We carried out a fire experiment and a capture-mark-recapture study to investigate habitat preferences, fire response and post-fire recovery in a vulnerable native Australian rodent, the pale field-rat. Fire treatments were used to approximate low intensity management burns (patchy fires) and high intensity wildfires (thorough fires). We used generalized linear mixed models to determine habitat associations, characterise the spatial distribution of surviving individuals immediately after fire, and investigate whether spatial recovery processes differ between fire types.

Pale field-rat populations were severely influenced by fire, with capture rate and the proportion of recaptures significantly declining with increasing fire extent. Furthermore, pale field-rat habitat preferences remained consistent before and after fire. Both pale field-rat populations and the vegetation completely recovered one year after fire, across both fire treatments. However, our findings suggest that spatial recovery processes may differ between fire treatments, with high survival suggesting that recovery was driven by *in situ* survivors within unburnt refuges after patchy fires, compared to high mortality suggesting recolonisation from outside the burnt area after thorough fires.

Our study suggests that pale field-rat persistence in the post-fire landscape is strongly dependent on the size and spatial pattern of fires, along with the presence of suitable remaining habitat (a combination of both long unburnt habitat and specific vegetation types). Thus, fire management strategies aiming to reduce the incidence of extensive wildfires and increase fine-grained patchiness will likely facilitate population recovery of vulnerable small mammal species through *in situ* survival within unburnt refuges.

Introduction

Ecological disturbance is a key driver of biodiversity, shaping the structure of communities, species distributions and population abundance (Turner 2010). Fire is a major disturbance agent globally and an important regulator of animal and plant populations, being particularly frequent in savanna landscapes (Bond and Keeley 2005, Harris et al. 2008). Altered fire regimes can change ecosystem structure and increase extinction risk for many species (Kelly et al. 2011, Lindenmayer et al. 2011). Thus, fire management has been recognised as a global issue and fundamental for biodiversity conservation, especially with wildfires predicted to increase in frequency and intensity in many ecosystems with climate change scenarios (Driscoll et al. 2010, Turner 2010).

A large proportion of tropical savannas are burnt each year by prescribed fire, making this biome one of the most actively fire managed in the world (Yates et al. 2008). Northern Australia has a long history of fire management, with prescribed burning by Aboriginal people carried out for millennia (Bowman 1998, Yibarbuk et al. 2001, Williams et al. 2003). Traditional fire practices likely resulted in fine-scale patterns of different fire histories across the landscape (Russell-Smith et al. 2003, Parr and Andersen 2006). However, since European colonisation and increasing pastoralism, there has been a shift towards much larger, frequent and intense wildfires occurring late in the dry season (Russell-Smith et al. 2003, Legge et al. 2011b). Thus, a core aim of contemporary fire management in northern Australia is to implement early dry season prescribed burning to reduce the extent and severity of destructive late dry season wildfires (Andersen et al. 2005, Legge et al. 2011b).

In Australia's monsoonal northern savannas, it has been well documented that frequent and extensive wildfires can have a profoundly negative impact on small mammal populations (Andersen et al. 2005, Legge et al. 2008, Radford et al. 2015). Furthermore, the interaction between altered fire regimes, grazing by introduced herbivores (such as cattle) and predation by feral cats (*Felis catus*) are now recognised as the key threats driving widespread small mammal declines across this region (Ziembicki et al. 2015). Fire reduces ground cover and the structural complexity of grass communities (McGregor et al. 2014, Leahy et al. 2016). Grazing also simplifies vegetation structure and can

exacerbate the detrimental effects of fire (Kutt and Woinarski 2007, Skroblin et al. 2014). This reduction in ground cover leaves animals exposed to predation, especially by feral cats, which prefer to hunt in both grazed and burnt habitat (McGregor et al. 2014, 2015, 2016). Furthermore, these exotic predators are capable of extirpating whole populations of native rodents in northern Australia (Frank et al. 2014).

While we are beginning to gain insight into the mechanisms underlying fire response in vulnerable species, research that focuses on understanding the post-fire recovery process will be particularly valuable. A key question yet to be answered is: how do populations recover after extensive, spatially homogeneous fires (i.e. wildfires) compared to early dry season management fires? In order to understand how post-fire recovery proceeds, it is important to determine the abundance and spatial distribution of survivors in relation to habitat variation and potential refuge areas (Banks et al. 2011). By understanding species-specific demographic traits, like post-fire survival and habitat requirements, we can make inferences about how population recovery mechanisms might change with different fire characteristics (for example, intensity, extent and fine-scale spatial arrangement) (Driscoll et al. 2010, Banks et al. 2011).

Motivated by the need to better understand the effects of the intensity and extent of fires on small mammal populations, we use a manipulative fire experiment to investigate fire response, fine-scale habitat requirements and population recovery in a vulnerable native rodent. We study the response of the pale field-rat (*Rattus tunneyi*), a vulnerable native rodent, to fire events of different spatial patterns and severity in north-western Australian savanna. We address four main questions:

i) *Do pale field-rats prefer particular types of vegetation and how do these differ in the level of cover they provide?*

   We compared pale field-rat abundance across the landscape to aerial mapping of vegetation. We then determined the level of cover each vegetation type provided at a range of heights important to pale field-rats and their ground predators.

ii) *How are different types of vegetation (especially those preferred by pale field-rats) affected by fires covering different spatial scales?*

We determined how the level of cover provided by the different types of vegetation changed from before to immediately after and one year after implementing a manipulative fire experiment.

iii) *How are pale-field rat captures and habitat preferences affected by fires covering different spatial scales?*

We trapped pale field-rats before, immediately after and one year after fire and investigated how abundance varied in response to the fine-scale extent of fire scars and different vegetation variables (determined through aerial mapping).

iv) *How is the proportion of recaptures affected by fires covering different spatial scales?*

We performed a capture-mark-recapture study of pale field-rats over three trapping sessions (before, immediately after and one year after fire). We investigated how the proportion of recaptures varied in response to fire extent, as an indication of pale field-rat survival. We combine these results with abundance data to make predictions about whether recovery was driven by *in situ* survivors or through recolonisation.

Methods

Study location

We conducted our study at the Mornington Wildlife Sanctuary (17.55°S, 126.17°E), in the savannas of the central Kimberley, in north-western Australia (Figure 1). The climate is monsoonal, with an average annual rainfall of 750 mm that falls mainly between December–February (Bureau of Meteorology). This 320,000 ha, former pastoral station is managed for conservation by the Australian Wildlife Conservancy (AWC). In 2004-2005, 40,300 ha of the sanctuary was destocked of introduced herbivores (primarily cattle) (Legge et al. 2011a). Mornington forms part of EcoFire, a collaborative early dry season prescribed burning project. This project has been managed by AWC since 2007, with the objective of reducing extensive, intense fires and increasing long-unburnt habitat (Legge et al. 2011b). Our study was carried out within the EcoFire and destocked area. The

vegetation of the study area is dominated by open savanna woodlands, with a sparse tree layer of eucalypts and a mix of tussock and hummock grasses.

Study species

The pale field-rat was once widespread across much of the Australian continent. Pale field-rats are now mostly restricted to the monsoonal tropics, but even here they have suffered a substantial range reduction of almost 30% in the last decade (Braithwaite and Griffiths 1996, Cole and Woinarski 2000, Start et al. 2012, Woinarski et al. 2014). Pale field-rats are known to be vulnerable to predation by feral cats, habitat degradation by introduced herbivores and inappropriate fire regimes (Braithwaite and Griffiths 1996, Legge et al. 2008, 2011a, Woinarski et al. 2010, 2011, 2014, Frank et al. 2014, Leahy et al. 2016).

Pale field-rats are associated with productive, riparian habitats and dense grassland near watercourses and seep areas (Braithwaite and Griffiths 1996, Braithwaite and Muller 1997, Taylor and Calaby 2004, Start et al. 2012). This species is mostly herbivorous, eating high nutrient stem and leaf material from a number of native grasses, as well as seeds, fruits, fungi and insects (to a lesser extent) (Braithwaite and Griffiths 1996). They make extensive, shallow burrows in sandy soils covering areas up to approximately 20 m^2 (Braithwaite and Griffiths 1996) and male home range size is larger than that of females (mean home range: 0.39 ha versus 0.09 ha, respectively) (Leahy et al. 2016). Breeding usually occurs in the first 6-8 months of each year, with the peak breeding period between March-April (Taylor and Calaby 2004). It is unlikely that many individuals survive beyond one breeding season, so generation length is likely 1-2 years (Woinarski et al. 2014).

Experimental design

In February 2015 – July 2016, we trapped pale field-rats across ten sites, that were part of a fire experiment that we implemented in 2015 (Figure 1). The sites were divided into experimental groups, each made up of a paired burnt and unburnt site. Paired sites followed the same ephemeral watercourse, although one site was on the Fitzroy River, which is permanently flowing. Each site was linear, approximately 1 km in length and 100 m – 1 km away from its pair. All sites had not been burnt for at least two years prior to

the current study, a typical fire return interval in northern Australian savannas (Yates et al. 2008).

In 2015, we performed a fire experiment, with five sites undergoing one of two fire treatments in a before-after-control-impact (BACI) design (Figure 1). We implemented low intensity fires at two sites, to approximate early dry season prescribed burns that affected less than 50% of the site. The low intensity fires occurred in cool conditions, during the evening in late March and early April, before the grass layer had cured (hereafter referred to as patchy fires). At a further three sites, we carried out high intensity burns that affected over 50% of the site, approximating late dry season unmanaged fires. These were implemented after the grass layer had cured, in late April and late May, during the middle of the day (hereafter referred to as thorough fires). The remaining five sites were unburnt controls. Treatment sites were paired with control sites within each group (with the exception of one stand-alone control, and one group of three sites where the original control was burnt during the experiment). While originally intended as a BACI design, fire treatments were more representative of a continuum rather than strict patchy and thorough categories. Therefore, for subsequent analyses, we use the percentage of the site that burnt to describe fire treatments.

Trapping Protocol

We trapped pale field-rats across three trapping sessions: immediately before, six weeks after, and one year after the fire treatments (Figure 1). For the second session, we trapped six weeks after fire, as previous research indicates that pale field-rats generally survive even intense fire events, however post-fire mortality is likely driven by predation, with predator activity highest from 2 – 6 weeks after fire (Leahy et al. 2016). At each site we set 100 steel Sherman Type A traps (30 x 10 x 8 cm) arranged in two transect lines approximately 30 – 40 m apart, with each transect comprising 50 traps spaced at 20 m intervals. We trapped at each site for approximately five nights (however, our analyses accounted for differences in the number of trap nights that arose due to inaccessibility or low captures in the late wet season). In total, we carried out 15,500 trap nights over the duration of our study. Paired control and treatment sites (within the same group) were trapped consecutively, within one week of each other (with the exception of the control site that was added as part of Group 3, which was trapped approximately 3 weeks

after the corresponding treatment sites). Traps were baited with rolled oats and peanut butter late in the afternoon, then checked and cleared before sunrise the next morning.

Trapping was carried out over three sessions (session 1: February – May 2015, session 2: May – July 2015 and session 3: March – June 2016), where captured animals were identified to species and sex. Pale field-rats were weighed, and implanted with a Trovan ID100 Midi-Chip (Microchips Australia Ltd, Melbourne; first and second trapping sessions only) for individual identification and marked with a white paint pen, so that recaptures could be immediately identified upon subsequent capture within each session. Females over 60 g and males over 65 g were classified as adults (Taylor and Calaby 2004, Leahy et al. 2016). All analyses were based on the number of pale field-rats captured that were unique to each session (i.e. excluding recaptures within sessions). However, recaptures between sessions were included in this measure of abundance. Pale field-rat abundance was offset by the total number of possible trap nights (the total number of trap nights corrected by subtracting the number of non-target captures and recaptures within each session). Thus, our response variable in subsequent analyses is the number of pale field-rat captures per available trap night (hereafter referred to as capture rate).

Aerial Mapping

Aerial photographs were taken from a helicopter specifically for this study, for vegetation and fire scar mapping. Photographs of study sites were taken before and immediately after all fire treatments. Photographs were georeferenced and stitched together in ARCMAP (Environmental System Research Institute Inc., Redlands, CA, USA). Mapping of vegetation (just before fire treatments) and fire scars (just after fire treatments) was carried out in a 50 m belt along each transect using aerial photographs and ground-truthing in QGIS (QGIS Development Team, Open Source Geospatial Foundation Project). The 50 m width was chosen to correspond with the home range size of pale field rats (Leahy et al. 2016). Vegetation types were categorised into one of four types of ground layer by the dominant grass species: mixed grasses, open grassland, riparian and tussock grass (Table 1). Creek lines were mapped, and scalds and roads (areas with little or no vegetation) were classified as 'other'. Vegetation categories were chosen to represent a range of different heights, easily mapped from aerial photographs (with the amount of

each vegetation type varying among sites; Appendix S1). Furthermore, these different vegetation types were roughly distributed along a gradient of increasing distance to creek lines, with riparian habitat adjacent to creeks, followed by tussock grass, open grassland and mixed grasses. Using the vegetation and fire scar maps, and ARCMAP's proximity toolkit (Environmental System Research Institute Inc., Redlands, CA, USA), we determined the area covered by different types of vegetation (before and immediately after fire) and the area burnt (immediately after fire) within a 20 m radius of each trap (corresponding to the distance between each trap point; Figure 2).

Vegetation surveys

The ground layer of open savanna woodland provides cover for small mammal species (Kutt and Woinarski 2007). Previous work has shown that a reduction in cover following fire resulted in a decline in pale field-rat abundance due to increased predation (Leahy et al. 2016). We assessed the level of cover provided by each of the four vegetation types within our sites, during the three trapping sessions (before, six weeks after and one year after our fire experiment). We established 84, 10 m^2 vegetation survey quadrats (two per vegetation type present at each site) that were marked for the duration of the study. Following Leahy et al. (2016), we estimated the structure provided by the ground layer at three height intervals (0–10 cm, 10–30 cm and 30–100 cm), using a modified version of the point height intercept technique, by estimating the number of stems intercepting a 1 m pole held perpendicular to the ground (MacArthur and MacArthur 1961, Spurr and Warburton 1991). Intercept estimates were given a score of 0, 1, 3, 5, 8, 10, 15 or 20. In order to capture the variation within each type of vegetation, all point estimates within each quadrat were used in subsequent analyses.

Statistical analyses

Overview

We fitted generalised linear mixed models (GLMMs) to investigate how pale field-rat capture rate changed over time and in response to fire. We investigated the total capture rate at each site, as well as fine-scale capture patterns at the trap level (specific details are provided below). We fitted poisson models (as our response variables were based on count data) in R (R Core Team 2017) using the *lme4* package (Bates et al. 2015). All models

were assessed for model fit by visually inspecting residuals for deviations from normality and uniformity using the *DHARMa* R package (Hartig 2017). Negative binomial models were fitted if poisson models were overdispersed (i.e. if the ratio of residual deviance to the degrees of freedom was greater than 1).

We conducted an exploratory spatial autocorrelation analysis to determine whether it was necessary to control for spatial autocorrelation of trap-level capture patterns in our GLMMs. We identified significant autocorrelation at the 40 m scale, decreasing to zero after 80 m (Appendix S2; Peakall & Smouse, 2006, 2012). Therefore, we calculated a spatial autocovariate from capture data, in the R package *spdep* (Bivand and Piras 2015) to take into account the spatial dependence in pale field-rat capture rates among neighbouring traps. We used an inverse weighting scheme with a neighbourhood radius of 80m, as this represents the spatial autocorrelation patterns seen in our exploratory correlograms (Appendix S2). We included the spatial autocovariate as a fixed effect in all trap-level models.

Do pale field-rats prefer particular types of vegetation and how do these differ in the level of cover they provide?

In order to determine whether pale field-rats had preferences for different types of vegetation, we investigated how the proportion of each vegetation type influenced pale field-rat capture rate at a local scale (20 m around each trap point) before the fire experiment was implemented. We fitted a negative binomial GLMM with 'capture rate' (at each trap) as the response variable. Fixed effects included the 'spatial autocovariate' and the 'percentage of the four different vegetation types within 20 m of each trap' (the 'global model'). 'Group' (within which paired treatment and control sites were nested) was included as a random term to account for the spatial proximity of paired sites.

We used model selection to compare all combinations of this 'global model' and to identify the most important vegetation variables influencing pale field-rat capture rate. We performed model selection using the *MuMIn* R package (Bartoń 2016), ranking models by the sample size corrected Akaike's information criterion (AIC_c), with smaller AIC_c values indicating that the relevant model was better supported (Burnham and

Anderson 2002). Models with AIC_c values that differed by less than 2 from the model with the lowest AIC_c were considered to be equivalent (Burnham and Anderson 2002).

Given the lack of a clear, top-ranked model (Akaike weight > 0.9) and consistent trends across fixed effects in the top models, we performed model averaging over all models within $\Delta AIC_c = 2$. We used the *MuMIn* R package (Bartoń 2016) to determine the relative importance of predictor variables (Holland and Bennett 2007, Symonds and Moussalli 2011). Model averaging produces weighted parameter estimates that are derived from multiple models, which in our case included all models that were within $AIC_c = 2$ of the top-ranked model (Burnham and Anderson 2002). We report the full model average, as this is more appropriate when the aim is to explore which particular variables have the strongest impact on the response variable (Burnham and Anderson 2002, Grueber et al. 2011). We also summed Akaike weights across all models (within the model averaging set) containing each specific variable. This measure indicates the importance of each variable, with larger values (max = 1) signifying more influential variables (Burnham and Anderson 2002).

We investigated differences in the level of ground cover among the different types of vegetation. We fitted negative binomial GLMMs for each height category, with the 'count of vegetation intercepts' (for a single point estimate) as the response variable and 'vegetation type' included as a fixed effect. Random effects included 'quadrat' nested within 'group' (due to spatial clustering of quadrats within groups and points within quadrats). We used the R packages lsmeans and multcomp (Hothorn et al. 2008, Lenth 2016) to make pairwise comparisons between all vegetation types (rather than just to the intercept).

How are different types of vegetation affected by fires covering different spatial scales?

To test how fire affects the level of cover provided by the different vegetation types, we used negative binomial GLMMs, with the 'count of vegetation intercepts' as the response variable. In order to determine how this measure of cover changed with the percentage of the site that was burnt, across the three trapping sessions, we fitted a three-way interaction, structured as 'session' x '% burnt' x 'vegetation type'. 'Quadrat' nested within 'group' were coded as random effects, due to spatial clustering and repeated sampling.

The '% burnt' variable was continuous, with control sites represented as 0% burnt. We used these '% burnt' values for session 1 (i.e. the percentage of the site that would eventually burn), under the assumption that there should be no relationship between this metric and the response variable before the fire experiment was implemented.

How are pale-field rat captures and habitat preferences affected by fires covering different spatial scales?

To investigate how pale field-rats are affected by fire, we first looked at capture rate at the site level. We fitted a negative binomial GLMM with 'capture rate' (site total) as the response variable. Fixed effects included 'session' and the percentage of the site that was burnt ('% burnt'), as well as an interaction between these variables ('session' x '% burnt'). 'Group' was included as a random effect. We then repeated this at the trap level to determine whether these patterns were also important over local scales (tens of metres). Trap-level models were fitted as poisson GLMMs, with 'capture rate' (at each trap) as the response variable. 'Session', the percentage of the area burnt within 20 m of each trap ('% burnt at trap'), an interaction between these variables ('session' x '% burnt at trap') and 'spatial autocovariate' were fitted as fixed effects. Random effects included 'trap' nested within 'group'. We ran all models for the total captures and separately for adults and juveniles. We also investigated whether this response differed between males and females and if fire affected the sex ratio. However, we found no change in sex ratio and no differences between the sexes and so do not report these results here.

Similar to our pre-fire analysis, we investigated how the proportion of each vegetation type influenced pale field-rat capture rate at a local scale (20 m around each trap point) six weeks after fire. However, in this case analyses only included treatment sites and represented the vegetation remaining after fire. A 'global' poisson GLMM was fitted with 'capture rate' (at each trap) as the response variable. The 'spatial autocovariate' and the 'percentage of the remaining vegetation types within 20 m of each trap' were included as fixed effects, with a random term of 'group'. Given the lack of a clear, stand-out model (Akaike weight > 0.9), we performed model averaging over models within $\Delta AIC_c = 2$.

How is the proportion of recaptures affected by fires covering different spatial scales?

We investigated how the proportion of recaptured pale field-rats varied between each trapping session and how this changed with fire, to make inferences about whether post-fire recovery stemmed from in situ survival or recolonisation. Recaptures were coded as a binary variable, with the number of recaptured individuals at each site relative to the total number of potential recaptures. We ran three binomial GLMMs for recaptures from session 1 – session 2, session 2 – session 3, and session 1 – session 3, with '% burnt' as the fixed effect and 'group' coded as the random effect.

We also calculated the mean maximum distance travelled between traps by all recaptured pale field-rats, both within and between sessions, to estimate home range movements and potential dispersal events.

Results

Do pale field-rats prefer particular types of vegetation and how do these differ in the level of cover they provide?

Across all sites, over three trapping sessions, we caught 951 unique pale field-rats (Figure 3).

Our model selection using pre-fire data indicated that the aggregate amount of both *riparian* and *tussock grass* vegetation within a 20 m radius of each trap were important predictors of pale field-rat captures. The top ranked model included a positive effect of these terms (AIC_c = 1393.781; Table 2). Model averaging revealed that pale field-rat capture rate increased as the amount of *tussock grass* and *riparian* vegetation surrounding each trap increased ($p<0.05$ and $p=0.052$, respectively; Table 3). Conversely, the aggregate amount of *mixed grasses* and *open grassland* vegetation surrounding each trap had a negative effect on pale field-rat captures ($p<0.05$ and $p=0.065$, respectively; Table 3). Summed Akaike weights across the top-ranked models were highest for *riparian* and *open grassland* vegetation (0.82 and 0.73, respectively), followed by *tussock grass* and *mixed grasses* (0.6 and 0.51, respectively; Table 3). This suggests that all four vegetation types were important predictors of pale field-rat capture rate, particularly *riparian* vegetation, which featured in all but one of the top-ranked models (Table 2).

The number of vegetation intercepts (intercept density) varied significantly among the different vegetation types at the three height categories measured (Figure 4; Appendix S3). Models featuring an effect of 'vegetation type' were well supported (0 – 10 cm: Null model ΔAIC_c = 43.779; 10 – 30 cm: Null model ΔAIC_c = 79.995; 30 – 100 cm: Null model ΔAIC_c = 45.643; Appendix S3). Intercept density was significantly greater in *tussock grass* compared to all other vegetation types, across all height categories (Figure 4; Appendix S4). *Riparian* intercept density was significantly lower than all vegetation types at the 0 – 10 cm and 10 – 30 cm height categories (with the exception of *open grassland* at 0 – 10 cm). However, *riparian* intercept density was similar to *mixed grasses* and *open grassland* at the 30 – 100 cm height category (Figure 4; Appendix S4). Intercept density did not differ significantly between *mixed grasses* and *open grassland* across all height categories, with one exception (intercept density was significantly greater in *mixed grasses* than in *open grassland* at 30 – 100 cm; Figure 4; Appendix S5).

How are different types of vegetation affected by fires covering different spatial scales?

The percentage of each treatment site that was burnt varied from 27 – 82% (Appendix S1). Models featuring main effects of 'session', '% burnt', 'vegetation type' and all two-way and three-way interactions between these variables were well supported (second-ranked models: 0-10 cm: ΔAIC_c = 117.554, 10-30 cm: ΔAIC_c = 163.628, 30 – 100 cm, ΔAIC_c = 69.689; Appendix S5). Predictions are summarised in Figure 5.

There was significant temporal variation in intercept density across all vegetation types and height categories, regardless of the fire treatment (Figure 5; Appendix S6). In unburnt (control) sites, intercept density was significantly higher in session 3 than in session 1 across all height categories (Appendix S6).

Immediately after the fire experiment (session 2), there was a significant negative effect of fire (the percentage of the site that was burnt) on intercept density in all vegetation types and height categories (Figure 5; Appendix S7). In preferred pale field-rat habitat, there were strong effects of fire on vegetation one year later. Compared to session 1, *riparian* intercept density was significantly higher one year after the fire experiment (session 3) at the 10 – 30 and 30 -100 cm height categories (while significantly

lower at the 0 -10 cm height category). Conversely, *tussock grass* intercept density was significantly lower in session 3 compared to session 1 as the percentage of the site that was burnt increased, at the 0 – 10 and 10 – 30 cm height intervals, although similar at the 30 – 100 cm category (Appendix S7).

How are pale-field rat captures and habitat preferences affected by fires covering different spatial scales?

Fire had a significant negative impact on capture rate, both over the broader scale (site) and local scale (trap), with strong support for all models featuring an effect of '% burnt', 'session' and an interaction between these two variables (Appendix S8-S9). Model findings were consistent between site and trap-level analyses. Here, we present results at the trap level only. However, site-level model summaries can be found in Appendix S10.

Pale field-rat capture rate was significantly higher in session 2 compared to session 1 and 3 in unburnt areas. However, there was a significant decline in pale field-rat capture rate in immediately after fire (session 2), as the percentage area burnt around the trap increased (Table 4; Figure 6). Predicted capture rate declined by 95% as the percentage area burnt around the trap increased from 0% to 100%.

Similar patterns were observed when considering separate models for adults and juveniles. In unburnt areas, capture rate was significantly higher in session 2, than in session 1 and 3, with this pattern strongest in juveniles (Table 4). As the percentage area burnt within a 20 m radius of the trap increased from 0% to 100%, both adult and juvenile capture rates decreased dramatically (by 98% and 93%, respectively) (Figure 6).

The percentage of the area that had previously burnt within a 20 m radius of the trap had no effect on pale field-rat capture rate one year later, in session 3 (Table 4; Figure 6; also found at the site level; Appendix S10). This was true for total captures, adults and juveniles. This suggests that pale field-rats recovered to similar numbers as found in unburnt areas (at the trap and site level), one year after the fire experiment was implemented (Table 4; Figure 6).

Pale field-rat habitat associations did not markedly change after fire. *Tussock grass* featured in all three of the top-ranked models, while *riparian* vegetation featured in all but one (Table 5). This resulted in summed Akaike weights of 1 and 0.714, respectively (Table 6). Pale field-rat capture rate increased significantly as the percentage of remaining *tussock grass* vegetation around the trap increased (Table 6). The positive effect of *riparian* vegetation on capture rate was not significant (p= 0.259). Interestingly, the significant negative effect of *mixed grasses* vegetation on capture rate was no longer present in the post-fire landscape (Table 6).

How is the proportion of recaptures affected by fires covering different spatial scales?

The number of recaptured individuals dropped dramatically over time, however, the percentage of the site that burnt had a significant, negative effect on the proportion of recaptures from before the fire experiment (session 1), to immediately after fire (session 1; Figure 7; Appendix S11). This model was well supported in session 1 – session 2, (null model: ΔAIC_c = 46.756; Appendix S12). However, the full models and null models were equivalent when investigating recaptures from session 2 – session 3 (Null ΔAIC_c = 0.812) and session 1 – session 3 (Null ΔAIC_c = 1.151; Appendix S12).

Overall, the mean maximum distance moved within sessions was 33 ± 4.6 m (maximum= 522.2 m). Males moved further than females, with a mean maximum distance of 52.8 ± 11.1 m compared to 20.7 ± 11.1 m, respectively. From session 1 to session 2, the mean maximum distance moved was 64.6 ± 31.8 m (maximum = 1882 m). From session 2 to session 3, recaptured individuals moved a mean maximum distance of 172.7 ± 99.3 m, (maximum = 1630.5 m). Finally, from session 1 to session 3, recaptured individuals moved a mean maximum distance of 86.7 ± 32.6 m (maximum = 236.4 m). In one case, an individual moved from a control site in session 1, to a patchily burnt site in session 2, while another individual moved from a control site in session 2 to a thoroughly burnt site in session 3.

Discussion

Prescribed burning is used globally across flammable landscapes and its role in conservation management is increasingly recognised (Driscoll et al. 2010, Fordyce et al. 2016). Here, we investigated the persistence of pale field-rats experiencing fires of differing spatial patterns and intensity. We determined the abundance and spatial distribution of surviving animals in relation to different habitat variables in an effort to understand the characteristics of vegetation and fire that are important for maintaining animals in the post-fire landscape. With this information, we make inferences about the mechanisms underlying the recovery process. Our study provides evidence that the short-term decline of pale field-rats due to fire is strongly dependent on fire size and spatial pattern, with the spatial distribution of suitable unburnt vegetation being critical to persistence within burnt landscapes immediately after fire.

Do pale field-rats prefer particular types of vegetation and how do these differ in the level of cover they provide?

The savannas of Australia's monsoonal tropics are characterised by broad scale uniformity. However, subtle variation in fire history, slope, geology, soil and moisture can strongly influence the abundance and distribution of animal and plant populations over more localised scales (Woinarski et al. 2005). Indeed, here we found strong associations with particular vegetation types over small scales of 0 – 20 m. At this scale, pale field-rat capture rate increased with the percentage of tussock grass and riparian vegetation.

Our results suggest that two microhabitat elements, cover and substrate, are important for the spatial distribution of pale field-rats across the landscape. Pale field-rat capture rate increased as the amount of tussock grass increased in the local area around our traps, as this vegetation type provided the highest level of cover. Intercept density in riparian vegetation, on the other hand, was significantly lower or similar to non-preferred vegetation types. However, the way in which we measured cover likely underestimated the complexity offered by *Passiflora foetida* (a climbing vine), which can be extremely dense along watercourses and grows over the top of other vegetation. The importance of dense vegetation cover and complex habitat is well recognised as an important feature for small, ground-dwelling mammals (Sutherland and Dickman 1999, Woinarski et al.

2004, Banks et al. 2011, Pereoglou et al. 2011, Fordyce et al. 2016). Ground-level vegetation structure can impact predator-prey interactions, with dense vegetation restricting predator movements and hunting success (McGregor et al. 2014, 2016). Furthermore, riparian vegetation tended to have much more substantial canopy cover (though, not measured here), which may be important for protecting pale field-rats from other known aerial predators such as owls and falcons (Leahy et al. 2016).

Secondly, mixed grasses and open grassland were associated with harder soils, or eroded, disturbed areas, whereas tussock grass and riparian vegetation were associated with loamy, friable soils in more productive, moist areas. Pale field-rats build complex burrows and are therefore likely restricted to these softer soils, closer to creek lines and seepage areas. Previous research has shown that pale field-rats show strong preferences for riparian habitats (Braithwaite and Griffiths 1996, Braithwaite and Muller 1997). Riparian zones are vital habitat for many species in northern Australia, as they support a disproportionately high number of species within these savanna landscapes and many species are almost completely restricted to these areas (Woinarski et al. 2005, Skroblin and Legge 2010). Thus, preserving this habitat, which is vulnerable to grazing, is an important conservation management goal (Tomkins and O'Reagain 2007, Skroblin et al. 2012).

How are different types of vegetation affected by fires covering different spatial scales?

Extreme climatic fluctuations over the seasons dominate the landscapes of Northern Australia (Woinarski et al. 2005). Our results reflected this seasonal variation, as we found significant temporal changes in vegetation intercept density, likely due in part to some grasses senescing during the dry season. However, fire was the most significant driver of change across all vegetation types, with intercept density declining markedly at all height intervals measured as the percentage of the site that was burnt increased. Furthermore, the decline in cover was much more dramatic in tussock grass, than at the other vegetation types analysed. The high connectivity of tussock grass means that this vegetation type burns readily once cured (Elliott et al. 2009, Bradstock 2010). Riparian vegetation has been identified as highly sensitive to fire, thus, the two types of vegetation associated with high pale field-rat captures either burn readily, or are strongly impacted by fire (Andersen et al. 2005).

Despite some changes in undergrowth, the level of cover provided by all vegetation types at 30 – 100 cm either did not change, or increased, one year after fire. Previous studies focusing on plants in northern Australia have shown that fire often has relatively little impact on many savanna vegetation communities. In fact, three key manipulative fire experiments in this region have shown that, while fire may reduce the amount of cover provided by perennial grasses in the short term, inter-annual variation in composition and diversity of the grass layer was mostly driven by rainfall (Bowman et al. 1988, Williams et al. 2003). Similarly, after experimental burning in the Okavango Delta, Botswana, Plavsic (2014) found that savanna vegetation was reduced immediately after fire, but almost completely recovered after one rainy season, and was indistinguishable from unburnt grassland after two to three rainy seasons. Thus, grass layer vegetation appears to be incredibly resilient to fire, and post-fire rainfall can drive rapid vegetation recovery even after intense fires.

How are pale-field rat captures and habitat preferences affected by fires covering different spatial scales?

Pale field-rat populations were significantly impacted immediately after fire, with captures strongly declining as the percentage of the site that was burnt increased. This decline was less pronounced after patchy fires than after thorough fires. Evidence suggests that, in general, fire events themselves do not cause direct mortality in small mammals (Sutherland and Dickman 1999, Griffiths and Brook 2014, Leahy et al. 2016). As shown in other studies, this decline in pale field-rat captures, particularly in thoroughly burnt sites, was most likely due to amplified predation and increased predation success after fire, facilitated by the removal of cover (Pardon et al. 2003, Fisher et al. 2014, McGregor et al. 2014, 2016, Leahy et al. 2016). While predation by native predators such as snakes, owls and dingoes is likely to be an important factor after fire, predation by feral cats may be the major driver of post-fire mortality in small mammals. This is because feral cats will target intense fire scars to take advantage of the newly open landscape for hunting (McGregor et al. 2014, 2016).

Similar to Leahy et al. (2016), we found that fire was not only important at the site level, but also over local scales, with the local area burnt around each trap a strong

predictor of pale field-rat captures. As the percentage area burnt around each trap increased, pale field-rat captures significantly decreased. Our prescribed burns were implemented during the breeding season, thus population declines may have been slightly buffered after patchy fires due to the recruitment of juveniles into the population (Leahy et al. 2016). Once the vegetation recovered, these factors may have allowed pale field-rat populations to quickly recover to pre-disturbance levels. Shilova and Tchabovsky (2009) found that rodent populations recovered quickly following pest control in Russia and the former USSR, due to the removal of dominant individuals supressing juvenile recruitment into the breeding population. Depending on species-specific life history traits and social structure, juveniles either matured earlier, or survived better and were able to gain access to previously occupied territories that were available post disturbance.

After patchy fires, we cannot say for certain whether the decline in pale field-rat capture rate was due to mortality or individuals avoiding burnt areas. Fordyce et al. (2016) found that bush rat movement pathways became more complex after a prescribed fire in south-eastern Australia. Essentially, bush rats tended to remain in unburnt vegetation, taking more convoluted paths to avoid patch edges. This was potentially the case after patchy fires, as more than half of the surrounding area within a 50m radius of each trap (roughly equivalent to a home range) remained unburnt, for the majority of traps in these sites. Alternatively, this was not an option for individuals in thoroughly burnt sites, as at least 75% of the trap area (within a distance equivalent to home range) was burnt for the majority of traps within these sites.

We found that pale field-rat habitat associations did not change substantially in the immediate post-fire landscape. Similarly, Diffendorfer et al. (2012) found a positive influence of nearby riparian or rocky substrates on the abundance of many small mammal species, with known preferences for these habitat characteristics, after a large wildfire in southern California. Furthermore, these vegetation associations affected long-term population recovery, highlighting the role of specific vegetation communities (rather than just unburnt vegetation as a whole) in supporting post-fire recovery in small mammals. However, in such dynamic and disturbance prone environments, it may be advantageous to have some degree of adaptability. For example, northern bobwhites (*Colinus virginianus*), a north American, ground dwelling bird, maintained high nest survival

despite frequent, extensive fires (Carroll et al. 2017). This was due to opportunistic and highly plastic behaviour, allowing this species to exploit a range of different nest substrates in the post-fire environment. These specific habitat preferences may be an important factor contributing to the pale field-rat's vulnerability to fire, suggesting that dense habitat close to watercourses is important for population persistence (and likely subsequent recovery) in the post-fire landscape.

It is likely that few survivors remained after the extensive, thorough fires, with mortality driving the post-fire declines in these sites. The proportion of recaptures also declined significantly as the percentage of the site that was burnt increased (from before, to immediately after fire). However, after patchy fires, post-fire declines may have reflected both mortality and/or avoidance of burnt areas, as there was plenty of available habitat within the scale of a home range. Leahy et al. (2015) found that individual pale field-rats did not shift territories if they were within burnt areas after fire. High home range fidelity after fire has been found across a number of Australian mammal species (Morris et al. 2011, MacGregor et al. 2013). In intensely burnt landscapes, this leaves animals with little shelter and thus vulnerable and exposed to predation. Therefore, the difference in the spatial distribution of survivors may indicate that these two fire types had different 'starting points for recovery' (Banks et al. 2011). These immediate post-fire patterns suggest that subsequent population recovery proceeded through different mechanisms; *in situ* survival versus recolonisation.

What are the potential mechanisms driving post-fire recovery and do they vary with the spatial scale of fire?

Post-fire abundance of small mammals has been strongly linked to the regeneration of vegetation and development of habitat structure (Monamy and Fox 2000, 2010, Fox et al. 2003). In general, structural characteristics of the environment are often a better predictor of fire response in small mammals than time since fire (Sutherland and Dickman 1999, Plavsic 2014, Swan et al. 2015). Much like the vegetation, pale field-rat populations completely recovered one year after fire. Both at the site level and at the local scale, there was no long-term effect of fire on pale field-rat captures or the vegetation. Thus, pale field-rat recovery likely followed the recovery of suitable vegetation in our experimental sites.

We did not find any long-term impact of fire on pale field-rat populations between fire treatments in our study. However, the scale of our experiment was such that suitable, unburnt habitat outside of the experimental plot was well within the dispersal capacity we measured for pale field-rats (up to 1882m). Thus, it is not surprising that populations equally recovered within both thorough and patchy fires. Regardless, few survivors remained in our sites immediately after thorough fires and the proportion of recaptures decreased as the percentage of the site that was burnt increased (from before to immediately after fire). This suggests that population recovery after thorough fires was likely driven by recolonisation from outside of the disturbed area.

The presence of refuges in disturbed landscapes creates the potential for population expansion from a number of dispersed nuclei when the required habitat once more becomes suitable (Turner et al. 1998, Banks et al. 2011, Robinson et al. 2013). Fine-grained refuges after patchy fires occurred over the scale of a pale field-rat home range, suggesting that more individuals survived after fire. Thus, post-fire recovery after patchy fires likely stemmed from *in situ* survivors in unburnt patches throughout the disturbed area. These recovery hypotheses warrant further investigation. Genetic analyses have the potential to help tease apart the different mechanisms underlying population recovery. In Chapter 5, we combine the demographic evidence presented here with a genetic investigation into these different modes of recovery.

Implications

The current objectives of many of the fire management programs in northern Australia focus on reducing the occurrence of extensive late dry season wildfires, increasing long unburnt vegetation, and increasing the patchiness of burns (Edwards et al. 2003, Legge et al. 2011b, Radford et al. 2015). Current research indicates that these objectives mitigate the threats to small mammals in the immediate post-fire landscape (Radford et al. *in prep*, Legge et al. 2011a, McGregor et al. 2014, Lawes et al. 2015a). Our results suggest that these management objectives are also likely to support the recovery of small mammal populations. However, a greater focus on the specific habitat requirements of vulnerable species will be important for ensuring these species persist in the post-fire landscape. Our research demonstrates the benefit of investigating fire response and

recovery mechanisms at the species level. A demographic understanding of the scale over which fires and unburnt refuges are important is vital for fire management strategies aiming to promote biodiversity (Driscoll et al. 2010). Furthermore, by understanding the importance of specific habitat attributes the operational goals of fire management programs can be more specific (Driscoll et al. 2010). Incorporating this knowledge into fire management strategies will become increasingly important as fire regimes change globally.

Tables and Figures

Table 1. Vegetation descriptions.

Vegetation type	Description	Dominant plant species	Soil
Mixed Grasses	Mixture of spinifex, tussock grasses and sparse annuals and perennials.	*Heterpogon contortus* *Eriachne obtusa* *Triodia bitextura* *Triodia wiseana*	Hard clay
Open Grassland	Annual grasses and sparse perennials. Often found in eroded areas and washouts.	*Xerochloa laniflora* *Eriachne obtusa*	Sand. Loose top layer, compacted underneath
Riparian	Densely covered with vines and thick, reedy plants with a much more apparent overstory.	*Passiflora foetida* *Mnesithea Formosa*	Moist, friable sand
Tussock Grass	Densely tufted perennials.	*Chrysopogon fallax* *Dichanthium fecundum*	Friable sand
Other	Roads, creek lines and scours (areas with no vegetation).	NA	Variable

Table 2. Model-selection results (for models within $\Delta AIC_c = 2$) for the effect of different vegetation variables on pale field-rat captures (per available trap night).

Variable groups	Model Structure	K	log(L)	AIC_c	ΔAIC_c	weight
% Veg type:	Rip + Tuss + Autocov	6	-690.850	1393.781	0.000	0.224
20 m trap radius	MG + OG + Rip + Autocov	7	-690.045	1394.197	0.416	0.182
	OG + Rip + Tuss + Autocov	7	-690.046	1394.200	0.420	0.181
	MG +OG + Autocov	6	-691.244	1394.569	0.788	0.151
	MG + OG + Rip + Tuss + Autocov	8	-689.655	1395.449	1.668	0.097

Included are the number of parameters (K), the log-likelihood values (log(L)), AIC_c values, AIC_c difference from the best model (ΔAICc) and Akaike weights. Autocov= Spatial Auotocovariate. This variable was held fixed for all model-selection groups. Vegetation codes are: Rip= Riparian; Tuss= Tussock grass; MG= Mixed grasses; OG= Open grassland.

Table 3. Model-average results across models within $\Delta AIC_c = 2$ of the top model, for the effect of different vegetation variables on pale field-rat captures (per available trap night).

Variable groups	Variable	Estimate	Std. error	Z value	p	Sum of weights
% Veg type:	(Intercept)	-3.470	0.559	6.201	<0.0001	-
20 m trap radius	%Rip	1.397	0.718	1.944	0.052	0.82
	%Tuss	0.931	0.413	2.251	<0.05	0.60
	%MG	-0.896	0.438	2.044	<0.05	0.51
	%OG	-1.260	0.682	1.845	0.065	0.73
	Autocov	3.369	0.518	6.495	<0.0001	1

Autocov= Spatial Auotocovariate. This variable was fixed during model averaging. Vegetation codes are: Rip= Riparian; Tuss= Tussock grass; MG= Mixed grasses; OG= Open grassland.

Table 4. Model summaries for the effect of session and percentage area burnt within a 20 m radius of each trap, on pale field-rat trap-level capture rate.

Individuals	Variable	Estimate	Std. Error	Z value	p
Total	S1 (Intercept)	-3.844	0.186	-20.651	<0.0001
	%Burnt	0.475	0.170	2.805	<0.01
	S2	0.699	0.083	8.391	<0.0001
	S3	-0.011	0.091	-0.119	0.905
	%Burnt x S2	-3.574	0.355	-10.075	<0.0001
	%Burnt x S3	0.010	0.200	0.052	0.959
	Autocov	1.908	0.191	9.967	<0.0001
	Random term	Variance	Standard Deviation		
	Trap within group	0.498	0.706		
	Group	0.097	0.312		
Adults	S1 (Intercept)	-4.273	0.221	-19.367	<0.0001
	%Burnt	0.303	0.220	1.376	0.169
	S2	0.595	0.116	5.114	<0.0001
	S3	0.110	0.119	0.917	0.359
	%Burnt x S2	3.372	0.441	7.652	<0.0001
	%Burnt x S3	-4.302	0.630	-6.825	<0.0001
	Autocov	-0.002	0.268	-0.008	0.993
	Random term	Variance	Standard Deviation		
	Trap within group	0.483	0.695		
	Group	0.128	0.358		
Juveniles	S1 (Intercept)	-4.379	0.255	-17.191	<0.0001
	%Burnt	0.555	0.227	2.444	<0.05
	S2	0.907	0.116	7.814	<0.0001
	S3	-0.220	0.138	-1.593	0.111
	%Burnt x S2	-3.416	0.428	-7.979	<0.0001
	%Burnt x S3	0.060	0.292	0.205	0.837
	Autocov	2.518	0.353	7.130	<0.0001
	Random term	Variance	Standard Deviation		
	Trap within group	0.607	0.779		
	Group	0.218	0.467		

Variable codes are: S1= Session 1 (immediately before fire); S2= Session 2 (six-weeks after fire); S3= Session 3 (one-year after fire); %Burnt= percentage of the area that was burnt within a 20 m radius of each trap. Autocov= Spatial Auotocovariate.

Table 5. Model-selection results (for models within ΔAIC_c = 2 and null models) for the effect of different habitat variables on pale field-rat captures (per available trap night).

Variable groups	Model structure	K	log(L)	AIC_c	ΔAIC_c	weight
% Remaining veg: 20 m trap radius	%Rip + %Tuss + Autocov	5	-130.990	272.102	0	0.278
	%Tuss + Autocov	4	-132.504	273.089	0.987	0.170
	%MG + %Rip + %Tuss + Autocov	6	-130.614	273.398	1.296	0.146
	Autocov (null model)	3	-135.797	277.641	5.540	0.017

Included are the number of parameters (K), the log-likelihood values (log(L)), AIC_c values, AIC_c difference from the best model (ΔAICc) and Akaike weights. Autocov= Spatial Auotocovariate. This variable was held fixed for all model-selection groups. Vegetation codes are: Rip= Riparian; Tuss= Tussock grass; MG= Mixed grasses.

Table 6. Model-averaged results investigating the effect of the percentage of remaining vegetation, with the percentage of area burnt within 20 m of each trap.

Variable groups	Variable	Estimate	Std. error	Z value	p	Sum of weights
% Remaining vegetation:	(Intercept)	-4.959	0.610	8.111	<0.0001	-
20 m trap radius	%Rip	3.168	2.801	1.130	0.259	0.714
	%Tuss	2.313	0.814	2.834	<0.01	1
	%MG	0.204	0.576	0.354	0.724	0.245
	Autocov	-0.503	3.477	0.144	0.885	1

Autocov= Spatial Auotocovariate. This variable was held fixed for all model selection groups. Vegetation codes are: Rip= Riparian; Tuss= Tussock grass; MG= Mixed grasses; OG= Open grassland.

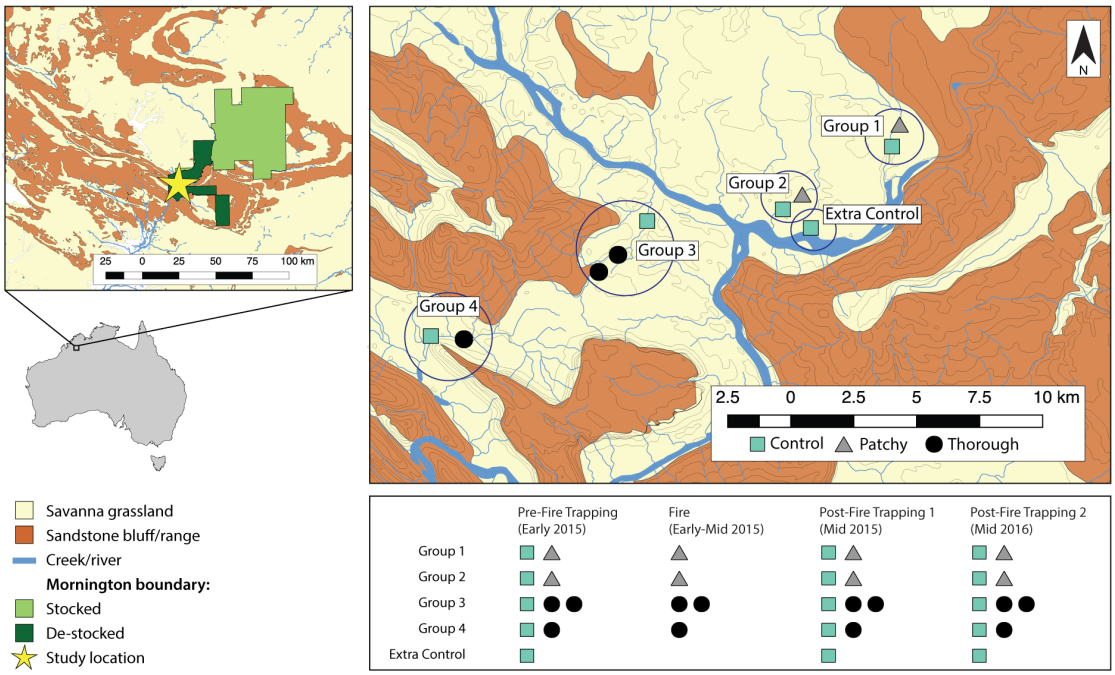

Figure 1. Map showing the study location, spatial arrangement of sites within groups and a schematic of the fire experiment (including the timing of each trapping session). The Mornington Wildlife Sanctuary property boundary is shown, with stocked and destocked boundaries referring to areas with and without introduced herbivores. All images were edited using Adobe Illustrator CC2014.

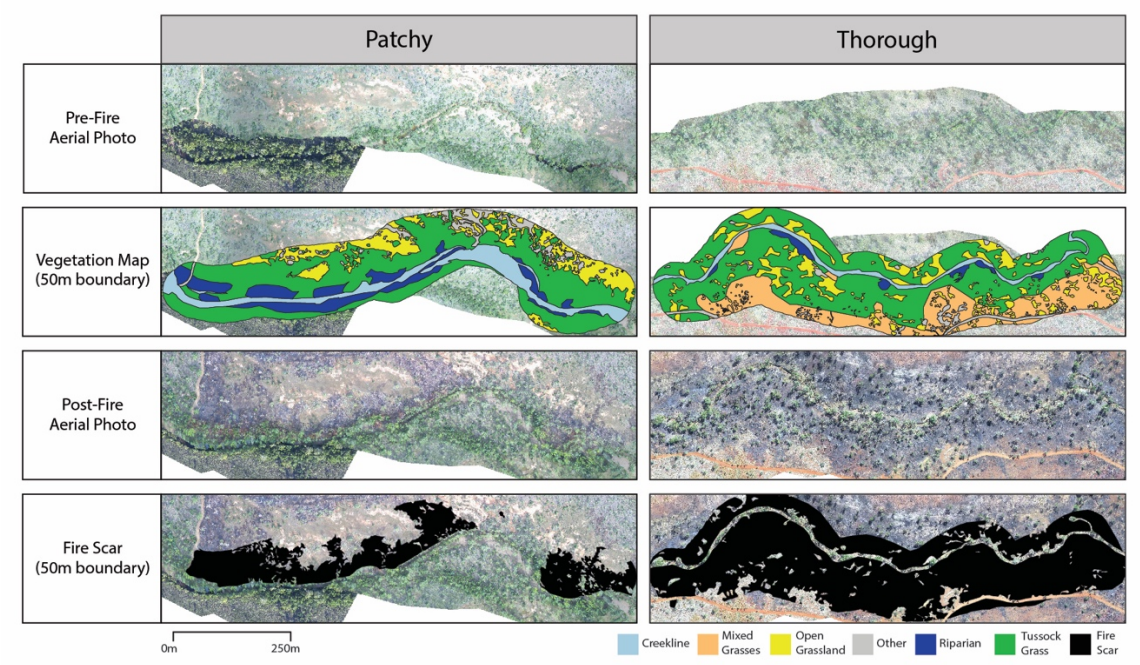

Figure 2. Aerial maps for one example (each) of a patchy and a thorough site.

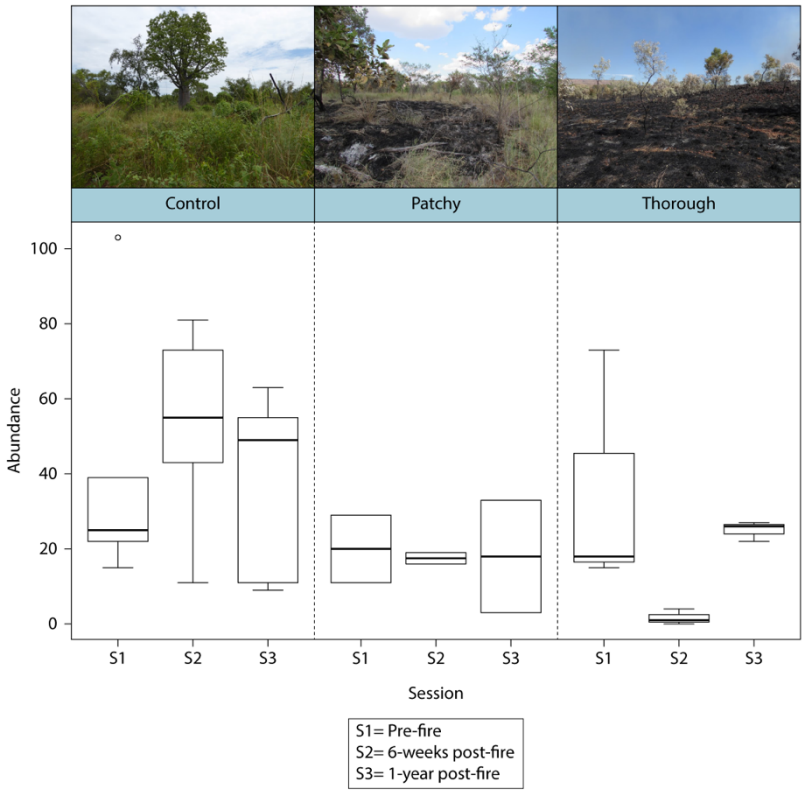

Figure 3. Boxplots showing patterns of abundance for pale field-rats over the three trapping sessions, across the different fire treatments. All plots were generated in GGPLOT2 (Wickham 2009).

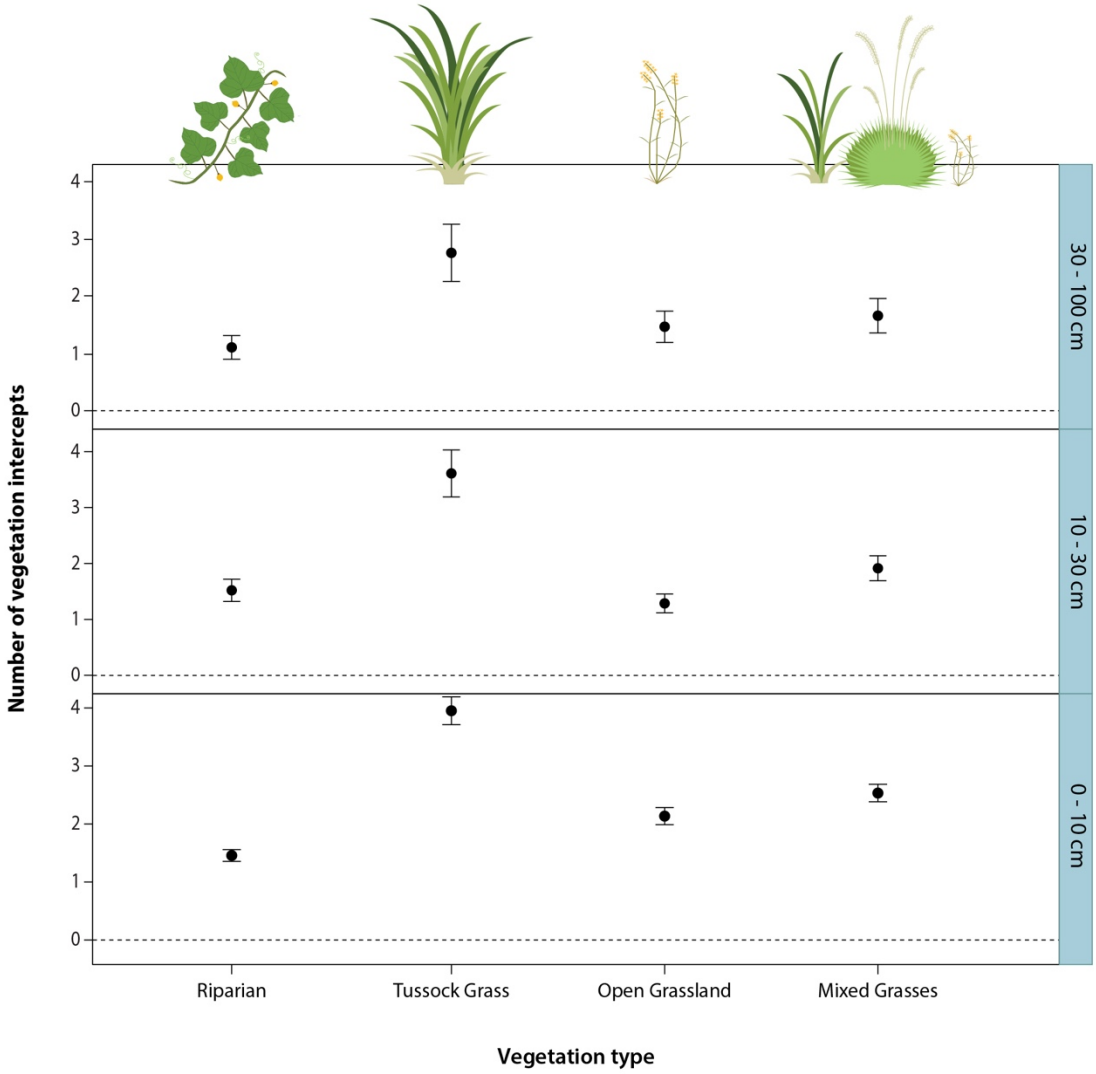

Figure 4. Model predictions (± standard error) of the number of vegetation intercepts (intercept density) across four different types of vegetation. Intercept density was measured over three height intervals, 0 – 10 cm, 10 – 30 cm and 30 – 100 cm.

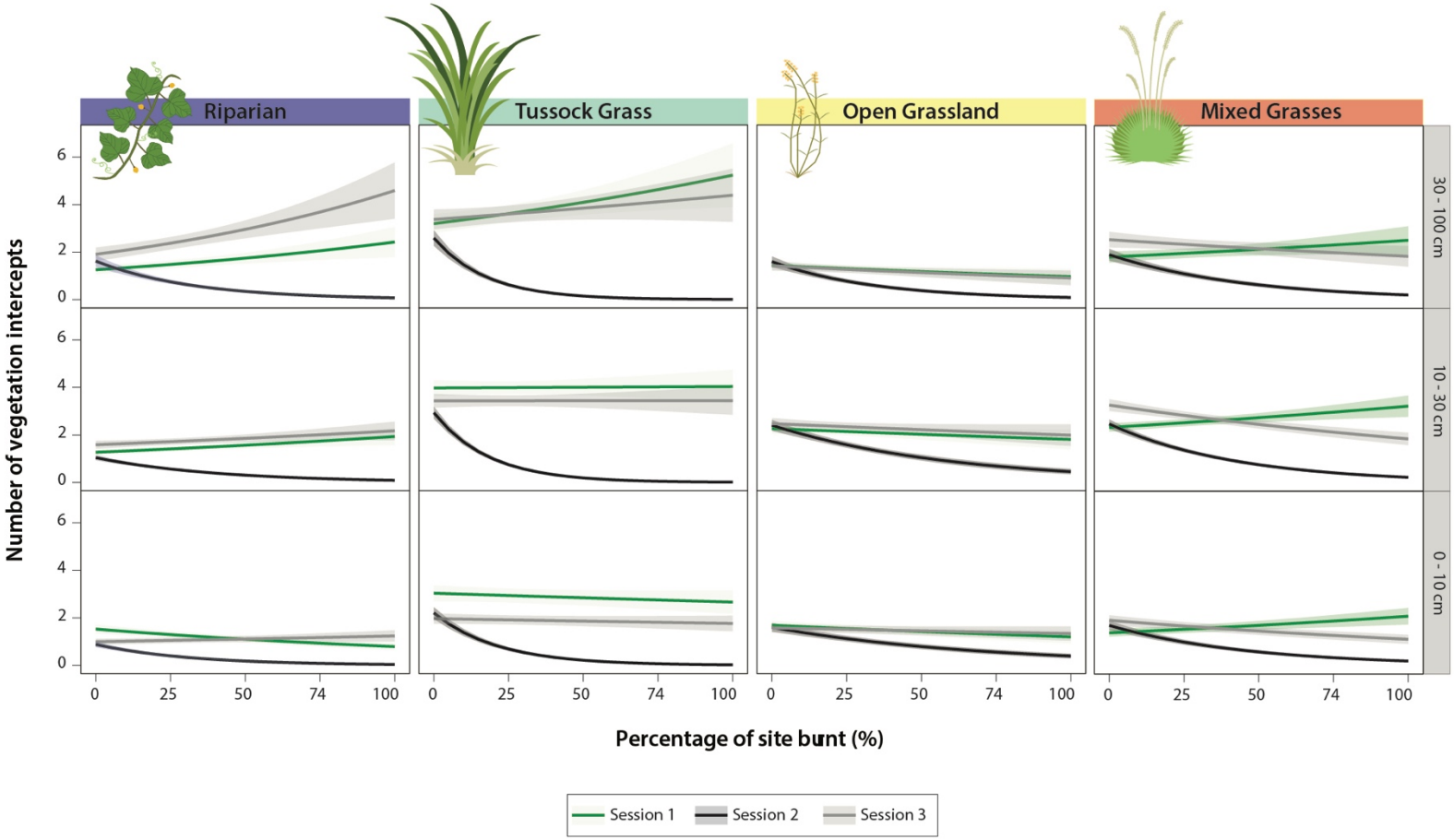

Figure 5. Model predictions (± standard error) for the effect of fire (percentage of the site that was burnt) on the number of vegetation intercepts (intercept density) at four different types of vegetation, over the three trapping sessions. Session 1 was carried out immediately before fire, session 2 was carried out six weeks after fire and session three took place one year after the experimental burns were implemented.

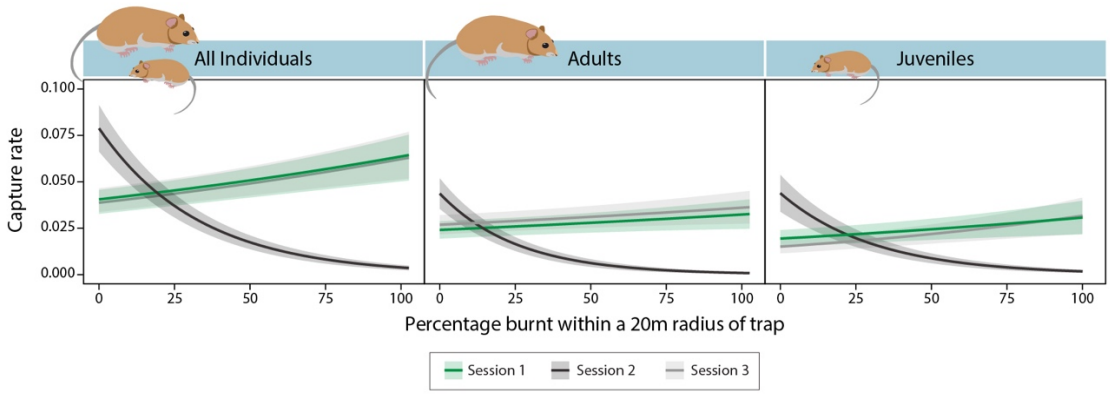

Figure 6. Model predictions (± standard error) for the local effect of fire (percentage of the area burnt within a 20 m radius of each trap) on pale field-rat capture rate per available trap night, over the three trapping sessions. Session 1 was carried out immediately before fire, session 2 was carried out six weeks after fire and session three took place one year after the experimental burns were implemented.

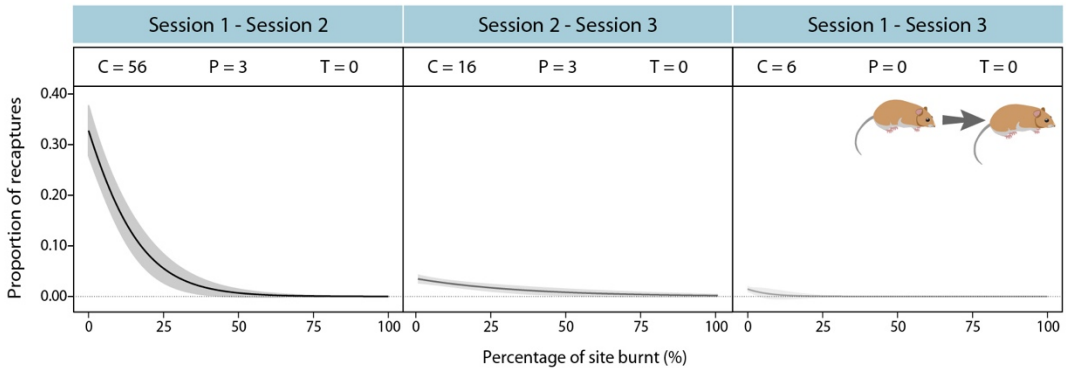

Figure 7. The effect of fire (percentage of the site that was burnt) on the predicted proportion of recapture (± standard error) between trapping sessions. Session 1 was carried out immediately before fire, session 2 was carried out six weeks after fire and session three took place one year after the experimental burns were implemented. Actual numbers of recaptured individuals are presented for control (C), patchy (P) and thorough (T) sites.

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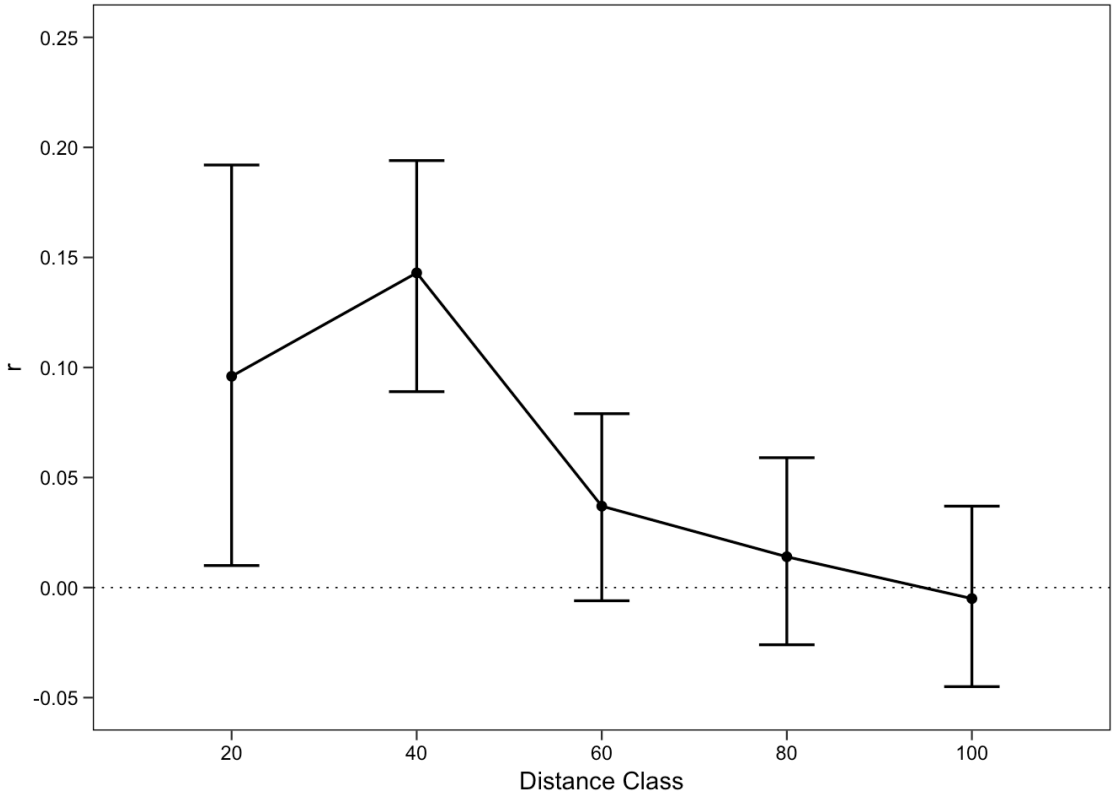
Appendix

Appendix S1. Percentage of each vegetation community within each site, and the percentage of each site that was burnt.

Site	Creek line (%)	Mixed Grasses (%)	Open Grassland (%)	Other (%)	Riparian (%)	Tussock (%)	Percentage Burnt (%)
RS08	12	0	9	4	16	59	0
RS09	14	0	17	6	13	50	32
RS10	13	17	11	5	1	53	0
RS11	16	22	10	4	0	48	27
RS12	5	23	16	4	2	50	82
RS13	6	31	11	5	5	41	80
RS14	8	37	19	5	5	26	0
RS15	10	29	9	12	5	36	51
RS16	14	34	3	10	6	33	0
RS17	6	33	9	5	3	43	0



Appendix S2. Correlogram of spatial autocorrelation of pale field-rat capture patterns.

Appendix S3. Model-selection for intercept density over different vegetation types, at the three height categories measured.

Height category	Model structure	K	log(L)	AIC_c	ΔAIC_c	weight
0 - 10 cm	Vegetation type	7	-7248.964	14513.96	0	1
	Random effects only (null model)	4	-7274.866	14557.74	43.779	0
10 - 30 cm	Vegetation type	7	-8467.884	16949.795	0	1
	Random effects only (null model)	4	-8510.890	17029.790	79.995	0
30 - 100 cm	Vegetation type	7	-7249.036	14512.099	0	1
	Random effects only (null model)	4	-7274.866	14557.741	45.643	0

Included are the number of parameters (K), the log-likelihood values (log(L)), AIC_c values, AIC_c difference from the best model (ΔAICc) and Akaike weights.

Appendix S4. Pairwise comparisons between vegetation intercept estimates for the different vegetation types, at the three height categories measured.

Height Category	Vegetation type 1	Vegetation type 2	Estimate	Standard error	Z value	p
0 - 10 cm	Mixed grasses	Open grassland	0.123	0.114	1.083	0.699
	Mixed grasses	Riparian	0.405	0.115	3.538	<0.01
	Mixed grasses	Tussock	-0.508	0.103	-4.914	<0.001
	Open grassland	Riparian	0.282	0.121	2.337	0.090
	Open grassland	Tussock	-0.631	0.112	-5.626	<0.001
	Riparian	Tussock	-0.913	0.112	-8.135	<0.001
10 - 30 cm	Mixed grasses	Open grassland	0.171	0.082	2.087	0.157
	Mixed grasses	Riparian	0.557	0.082	6.759	<0.001
	Mixed grasses	Tussock	-0.447	0.075	-5.993	<0.001
	Open grassland	Riparian	0.385	0.089	4.310	<0.001
	Open grassland	Tussock	-0.618	0.083	7.475	<0.001
	Riparian	Tussock	-1.003	0.083	12.106	<0.001
30 - 100 cm	Mixed grasses	Open grassland	0.395	0.152	2.588	0.047
	Mixed grasses	Riparian	0.229	0.151	1.514	0.428
	Mixed grasses	Tussock	-0.632	0.139	-4.554	<0.001
	Open grassland	Riparian	-0.165	0.159	-1.040	0.726
	Open grassland	Tussock	-1.026	0.150	-6.830	<0.001
	Riparian	Tussock	-0.861	0.148	-5.822	<0.001

Appendix S5. Model-selection for the effect of vegetation type, session and percentage of the site that was burnt on intercept density, across the three height categories measured.

Height Category	Model structure	K	log(L)	AIC_c	ΔAIC_c	weight
0 - 10 cm	Session x vegetation type x % burnt	21	-13548.510	27139.140	0	1
	Session x % burnt + session x vegetation type	15	-13613.320	27256.690	117.554	0
	Random effects only (null model)	4	-14188.170	28384.350	1245.218	0
10 - 30 cm	Session x vegetation type x % burnt	21	-15853.120	31748.340	0	1
	Session x % burnt + session x vegetation type	15	-15940.960	31911.970	163.628	0
	Random effects only (null model)	4	-16826.080	33660.160	1911.814	0
30 - 100 cm	Session x vegetation type x % burnt	21	-15299.480	30641.080	0	1
	Session x % burnt + session x vegetation type	15	-15340.360	30710.770	69.689	0
	Random effects only (null model)	4	-16185.370	32378.740	1737.658	0

Included are the number of parameters (K), the log-likelihood values (log(L)), AIC_c values, AIC_c difference from the best model (ΔAICc) and Akaike weights. Only the best model, second best model and null model are presented, due to overwhelming support for the best model (w>0.9 and ΔAIC_c of the second-ranked model >10)

Appendix S6. Model summaries for the effect of vegetation community, session and percentage of the site that was burnt on intercept density, across the three height categories measured.

Height Category	Variable	Estimate	Standard error	Z value	p
0 - 10 cm	S1:Mixed grasses (Intercept)	0.330	0.112	2.951	<0.01
	S2	0.200	0.062	3.237	<0.01
	S3	0.321	0.062	5.178	<0.0001
	% Burnt	0.407	0.181	2.244	<0.05
	Open grassland	0.172	0.115	1.496	0.135
	Riparian	0.091	0.121	0.752	0.452
	Tussock grass	0.776	0.105	7.400	<0.0001
	S2 x % burnt	-2.539	0.179	-14.191	<0.0001
	S3 x % burnt	-0.946	0.143	-6.600	<0.0001
	S2 x open grassland	-0.298	0.095	-3.146	<0.01
	S3 x open grassland	-0.398	0.095	-4.213	<0.0001
	S2 x riparian	-0.745	0.106	-7.009	<0.0001
	S3 x riparian	-0.744	0.102	-7.297	<0.0001
	S2 x tussock grass	-0.524	0.086	-6.121	<0.0001
	S3 x tussock grass	-0.754	0.085	-8.887	<0.0001
	% Burnt x open grassland	-0.706	0.300	-2.351	<0.05
	% Burnt x riparian	-1.062	0.273	-3.885	<0.0001
	% Burnt x tussock grass	-0.531	0.250	-2.124	<0.05
	S2 x % burnt x open grassland	1.553	0.293	5.300	<0.0001
	S3 x % burnt x open grassland	1.144	0.253	4.527	<0.0001
	S2 x % burnt x riparian	-0.009	0.367	-0.025	0.980
	S3 x % burnt x riparian	1.813	0.240	7.548	<0.0001
	S2 x % burnt x tussock grass	-1.984	0.341	-5.827	<0.0001
	S3 x %Burnt x tussock grass	0.967	0.208	4.652	<0.0001
		Variance	Standard Deviation		
	Random term: Quadrat within group	0.050	0.223		
	Random term: group	0.034	0.184		

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Height Category	Variable	Estimate	Standard error	Z value	p
10 - 30 cm	S1:Mixed grasses (Intercept)	0.850	0.076	11.158	<0.0001
	S2	0.059	0.053	1.121	0.262
	S3	0.338	0.052	6.513	<0.0001
	% Burnt	0.324	0.174	1.865	0.062
	Open grassland	-0.047	0.119	-0.390	0.697
	Riparian	-0.607	0.125	-4.866	<0.0001
	Tussock grass	0.532	0.109	4.861	<0.0001
	S2 x % burnt	-2.664	0.152	-17.521	<0.0001
	S3 x % burnt	-0.893	0.119	-7.518	<0.0001
	S2 x open grassland	0.008	0.083	0.094	0.925
	S3 x open grassland	-0.245	0.081	-3.024	<0.01
	S2 x riparian	-0.256	0.095	-2.697	<0.01
	S3 x riparian	-0.117	0.089	-1.316	0.188
	S2 x tussock grass	-0.366	0.074	-4.910	<0.0001
	S3 x tussock grass	-0.484	0.072	-6.708	<0.0001
	% Burnt x open grassland	-0.519	0.314	-1.654	0.098
	% Burnt x riparian	0.094	0.282	0.332	0.740
	% Burnt x tussock grass	-0.309	0.263	-1.172	0.241
	S2 x % burnt x open grassland	1.198	0.258	4.648	<0.0001
	S3 x % burnt x open grassland	0.890	0.214	4.157	<0.0001
	S2 x % burnt x riparian	-0.208	0.287	-0.725	0.469
	S3 x % burnt x riparian	0.788	0.198	3.985	<0.0001
	S2 x % burnt x tussock grass	-2.807	0.327	-8.576	<0.0001
	S3 x %Burnt x tussock grass	0.880	0.174	5.063	<0.0001
		Variance	Standard Deviation		
	Random term: Quadrat within Group	6.67E-02	2.58E-01		
	Random term: Group	4.16E-09	6.45E-05		

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Height Category	Variable	Estimate	Standard error	Z value	p
30 - 100 cm	S1:Mixed grasses (Intercept)	0.596	0.129	4.632	<0.0001
	S2	0.052	0.066	0.785	0.433
	S3	0.339	0.065	5.182	<0.0001
	% Burnt	0.329	0.295	1.114	0.265
	Open grassland	-0.275	0.202	-1.362	0.173
	Riparian	-0.369	0.207	-1.784	0.074
	Tussock grass	0.567	0.185	3.061	<0.01
	S2 x % burnt	-2.509	0.183	-13.730	<0.0001
	S3 x % burnt	-0.651	0.147	-4.426	<0.0001
	S2 x open grassland	0.066	0.106	0.623	0.533
	S3 x open grassland	-0.343	0.105	-3.275	<0.01
	S2 x riparian	0.203	0.109	1.864	0.062
	S3 x riparian	0.079	0.105	0.749	0.454
	S2 x tussock grass	-0.263	0.093	-2.829	<0.01
	S3 x tussock grass	-0.285	0.091	-3.144	<0.01
	% Burnt x open grassland	-0.652	0.530	-1.231	0.218
	% Burnt x riparian	0.329	0.472	0.698	0.485
	% Burnt x tussock grass	0.166	0.447	0.371	0.711
	S2 x % burnt x open grassland	-0.008	0.355	-0.023	0.981
	S3 x % burnt x open grassland	0.589	0.276	2.132	<0.05
	S2 x % burnt x riparian	-1.247	0.320	-3.893	<0.0001
	S3 x % burnt x riparian	0.873	0.229	3.816	0.000
	S2 x % burnt x Tussock Grass	-3.711	0.387	-9.576	<0.0001
	S3 x %Burnt x Tussock Grass	0.421	0.214	1.966	<0.05
		Variance	Standard Deviation		
	Random term: Quadrat within Group	2.15E-01	4.63E-01		
	Random term: Group	<0.0001	<0.0001		

Appendix S7. Pairwise comparisons between intercept density across all three-way interactions involving session, vegetation type and the percentage of the site that was burnt.

Height Category	Comparison 1	Comparison 2	Estimate	Std. Error	Z value	p
0-10 cm	S1 x % burnt x Mixed grasses	S2 x % burnt x Mixed grasses	0.468	0.053	8.839	<0.001
	S1 x % burnt x Mixed grasses	S3 x % burnt x Mixed grasses	-0.072	0.048	-1.515	0.917
	S2 x % burnt x Mixed grasses	S3 x % burnt x Mixed grasses	-0.540	0.054	-10.082	<0.001
	S1 x % burnt x Open grassland	S2 x % burnt x Open grassland	0.357	0.062	5.810	<0.001
	S1 x % burnt x Open grassland	S3 x % burnt x Open grassland	0.025	0.058	0.428	1.000
	S2 x % burnt x Open grassland	S3 x % burnt x Open grassland	-0.333	0.062	-5.386	<0.001
	S1 x % burnt x Riparian	S2 x % burnt x Riparian	1.215	0.082	14.832	<0.001
	S1 x % burnt x Riparian	S3 x % burnt x Riparian	0.194	0.060	3.244	<0.05
	S2 x % burnt x Riparian	S3 x % burnt x Riparian	-1.021	0.083	-12.293	<0.001
	S1 x % burnt x Tussock grass	S2 x % burnt x Tussock grass	1.514	0.073	20.844	<0.001
	S1 x % burnt x Tussock grass	S3 x % burnt x Tussock grass	0.427	0.046	9.310	<0.001
	S2 x % burnt x Tussock grass	S3 x % burnt x Tussock grass	-1.087	0.075	-14.566	<0.001
10-30 cm	S1 x % burnt x Mixed grasses	S2 x % burnt x Mixed grasses	0.642	0.045	14.253	<0.01
	S1 x % burnt x Mixed grasses	S3 x % burnt x Mixed grasses	-0.103	0.040	-2.574	0.235
	S2 x % burnt x Mixed grasses	S3 x % burnt x Mixed grasses	-0.745	0.045	-16.434	<0.01
	S1 x % burnt x Open grassland	S2 x % burnt x Open grassland	0.319	0.054	5.904	<0.01
	S1 x % burnt x Open grassland	S3 x % burnt x Open grassland	-0.092	0.050	-1.841	0.734
	S2 x % burnt x Open grassland	S3 x % burnt x Open grassland	-0.411	0.054	-7.665	<0.01
	S1 x % burnt x Riparian	S2 x % burnt x Riparian	0.953	0.068	14.102	<0.01
	S1 x % burnt x Riparian	S3 x % burnt x Riparian	-0.193	0.053	-3.666	<0.01
	S2 x % burnt x Riparian	S3 x % burnt x Riparian	-1.146	0.067	-17.219	<0.01
	S1 x % burnt x Tussock grass	S2 x % burnt x Tussock grass	1.747	0.073	23.863	<0.01
	S1 x % burnt x Tussock grass	S3 x % burnt x Tussock grass	0.150	0.039	3.803	<0.01
	S2 x % burnt x Tussock grass	S3 x % burnt x Tussock grass	-1.597	0.074	-21.695	<0.01
30-100 cm	S1 x % burnt x Mixed grasses	S2 x % burnt x Mixed grasses	0.609	0.055	11.053	<0.001
	S1 x % burnt x Mixed grasses	S3 x % burnt x Mixed grasses	-0.167	0.050	-3.354	<0.05
	S2 x % burnt x Mixed grasses	S3 x % burnt x Mixed grasses	-0.776	0.055	-14.092	<0.001
	S1 x % burnt x Open grassland	S2 x % burnt x Open grassland	0.545	0.077	7.078	<0.001
	S1 x % burnt x Open grassland	S3 x % burnt x Open grassland	0.020	0.066	0.306	1.000
	S2 x % burnt x Open grassland	S3 x % burnt x Open grassland	-0.525	0.077	-6.819	<0.001
	S1 x % burnt x Riparian	S2 x % burnt x Riparian	0.734	0.073	10.083	<0.001
	S1 x % burnt x Riparian	S3 x % burnt x Riparian	-0.476	0.060	-7.916	<0.001
	S2 x % burnt x Riparian	S3 x % burnt x Riparian	-1.209	0.072	-16.900	<0.001
	S1 x % burnt x Tussock grass	S2 x % burnt x Tussock grass	1.849	0.085	21.774	<0.001
	S1 x % burnt x Tussock grass	S3 x % burnt x Tussock grass	0.007	0.049	0.139	1.000
	S2 x % burnt x Tussock grass	S3 x % burnt x Tussock grass	-1.842	0.085	-21.660	<0.001

Appendix S8. Model-selection for the effect of session and percentage of the site that was burnt, on pale field-rat capture rate for different groups of individuals.

Individuals	Model structure	K	log(L)	AICc	ΔAICc	weight
Total	Session x % burnt	8	-119.694	262.245	0	1
	% Burnt	4	-141.408	292.416	30.171	0
	Random effects only (null model)	3	-144.336	295.595	33.350	0
Adults	Session x % burnt	8	-97.489	217.835	0	1
	% Burnt	4	-122.554	254.708	36.873	0
	Session + % burnt	6	-122.053	259.759	41.924	0
	Random effects only (null model)	3	-128.574	264.071	46.236	0
Juveniles	Session x % burnt	8	-104.639	232.135	0	0.990
	Random effects only (null model)	3	-118.673	244.270	12.135	0.005

Included are the number of parameters (K), the log-likelihood values (log(L)), AIC_c values, AIC_c difference from the best model (ΔAICc) and Akaike weights. Only the best model, second best model and null model are presented, due to overwhelming support for the best model (w>0.9 and ΔAIC_c of the second-ranked model >10).

Appendix S9. Model-selection for the effect of session and percentage of the area that was burnt within a 20m radius of each trap, on pale field-rat trap-level captures, for different groups of individuals.

Individuals	Model structure	K	log(L)	AIC_c	ΔAIC_c	weight
Total	Session x % burnt + autocov	9	-2092.297	4202.654	0	1
	Session + % burnt + autocov	7	-2178.122	4370.281	167.627	0
	% Burnt + autocov	6	-2180.428	4372.884	170.231	0
	Session + autocov	5	-2184.768	4379.557	176.903	0
	Autocov (null model)	4	-2186.889	4381.792	179.138	0
Adults	Session x % burnt + autocov	9	-1430.005	2878.071	0.000	1
	% Burnt + autocov	5	-1478.502	2967.024	88.953	0
	Session + % burnt + autocov	7	-1477.937	2969.911	91.841	0
	Autocov (null model)	4	-1481.471	2970.956	92.885	0
Juveniles	Session x % burnt + autocov	9	-1307.144	2632.347	0	1
	Session + % burnt + autocov	7	-1353.868	2721.772	89.425	0
	Session + autocov	6	-1355.970	2723.968	91.621	0
	% Burnt + autocov	5	-1369.615	2749.249	116.902	0
	Autocov (null model)	4	-1371.314	2750.640	118.294	0

Included are the number of parameters (K), the log-likelihood values (log(L)), AIC_c values, AIC_c difference from the best model (ΔAICc) and Akaike weights. Only the best model, second best model and null model are presented, due to overwhelming support for the best model (w>0.9 and ΔAIC_c of the second-ranked model >>10). Autocov= Spatial Auotocovariate. This variable was held fixed for all model-selection groups.

Appendix S10. Model summaries for the effect of session and percentage of the site that was burnt on pale Field-rat captures per available trap night.

Individuals	Variable	Estimate	Std. Error	Z value	p
Total	S1 (Intercept)	-2.699	0.348	-7.748	<0.0001
	%Burnt	-0.128	0.552	-0.232	0.817
	S2	0.777	0.284	2.739	<0.01
	S3	-0.126	0.276	-0.457	0.647
	%Burnt x S2	-5.049	1.015	-4.977	<0.0001
	%Burnt x S3	0.626	0.676	0.927	0.354
	Random term	Variance	Standard Deviation		
	Group	0.402	0.634		
Adults	S1 (Intercept)	-3.192	0.315	-10.127	<0.0001
	%Burnt	-0.393	0.491	-0.800	0.424
	S2	0.603	0.242	2.490	<0.05
	S3	-0.010	0.237	-0.043	0.966
	%Burnt x S2	-5.983	1.162	-5.147	<0.0001
	%Burnt x S3	0.538	0.616	0.874	0.382
	Random term	Variance	Standard Deviation		
	Group	0.345	0.587		
Juveniles	S1 (Intercept)	-3.546	0.448	-7.916	<0.0001
	%Burnt	0.186	0.761	0.244	0.807
	S2	1.068	0.391	2.733	<0.01
	S3	-0.325	0.387	-0.841	0.400
	%Burnt x S2	-4.713	1.276	-3.692	<0.0001
	%Burnt x S3	0.755	0.924	0.817	0.414
	Random term	Variance	Standard Deviation		
	Group	0.606	0.779		

Variable codes are: S1= Session 1 (immediately before fire); S2= Session 2 (six-weeks after fire); S3= Session 3 (one-year after fire); %Burnt= percentage of the site that was burnt.

Appendix S11. Model summary for recaptured pale field-rats between sessions, with the percentage of the site that was burnt.

Sessions	Variable	Estimate	Std. error	Z value	p
S1 – S2	Intercept	-0.719	0.225	-3.197	<0.001
	%SiteBurnt	-8.453	2.147	-3.937	<0.0001
		Variance	Standard Deviation		
	Random term: Group	0.09	0.3		
S2 – S3	Intercept	-4.210	0.414	-10.166	<0.0001
	%SiteBurnt	-14.169	26.874	-0.527	0.598
		Variance	Standard Deviation		
	Random term: Group	<0.0001	<0.0001		
S1 – S3	Intercept	-3.325	0.267	-12.436	<0.0001
	%SiteBurnt	-2.937	1.944	-1.511	0.131
		Variance	Standard Deviation		
	Random term: Group	<0.0001	<0.0001		

Appendix S12. Model-selection for recaptured pale field-rats between sessions, with the percentage of the site that was burnt.

Sessions	Model structure	K	log(L)	AIC_c	ΔAIC_c	weight
S1 - S2	%SiteBurnt	3	-17.527	45.054	0	1
	Random effects only (null model)	2	-43.048	91.809	46.756	0
S2 - S3	Random effects only (null model)	2	-13.528	32.771	0	0.6
	%SiteBurnt	3	-11.792	33.583	0.812	0.4
S1 - S3	Random effects only (null model)	2	-7.761	21.236	0	0.64
	%SiteBurnt	3	-6.193	22.386	1.151	0.36

Included are the number of parameters (K), the log-likelihood values (log(L)), AICc values, AICc difference from the best model (ΔAICc) and Akaike weights.

Chapter 5

Genetic evidence suggests mechanisms for post-fire recovery differ with the extent of experimental fire

Abstract

While a large body of research has concentrated on fire response in small mammals, studies rarely focus on the post-fire recovery process. Furthermore, no study has yet combined demographic and genetic evidence to understand how small mammal populations recover after fire in northern Australia. In particular, knowledge on how the recovery process might differ between extensive, threatening fires (thorough fires), compared to lower intensity management fires (patchy fires) will be critical if we are to facilitate the recovery of small mammals across the landscape.

We used genetic analyses to investigate population recovery in a native Australian rodent, the pale field-rat (*Rattus tunneyi*) following a manipulative fire experiment in the Kimberley region of Western Australia. We explored how genetic patterns change from before the fire experiment, to one year after fires of differing spatial scales and intensity (patchy versus thorough fires).

By testing a number of genetic and demographic predictions relating to the different recovery hypotheses, our findings suggest that *in situ* survival drives population recovery after patchy fires, compared to recolonisation from source populations located along creek lines after thorough fires. Furthermore, while male dispersal appeared relatively constant, females potentially exhibit context dependent 'dispersal polymorphism', with dispersal patterns changing depending on the extent of experimental fires. Thus, fire management strategies that support *in situ* survival and maintain suitable recolonisation sources and connectivity will be critical for facilitating post-fire recovery in many small mammal populations.

Introduction

Analyses of genetic diversity and its partitioning among individuals and populations can provide insights into reproduction, mortality, dispersal and habitat connectivity in animal populations (Holderegger et al. 2006). While genetic analyses have been successfully applied to many aspects of ecology and conservation biology, they are underutilised in disturbance ecology (Allendorf et al. 2010, Storfer et al. 2010, Banks et al. 2013). In particular, there has been limited application of genetic research to inform fire management for biodiversity conservation. Fire can directly impact animal populations through increasing mortality and forcing emigration, or indirectly through changing habitat or nutrient availability (Zwolak 2009, Bradstock et al. 2012). Therefore, in combination with demographic evidence, genetic data could be used to determine the mechanisms by which animal populations recover after fire. This knowledge is vital for fire management, as these mechanisms have implications for the effects of fire regimes on population persistence (Romme et al. 1998, Davies et al. 2016).

Investigating population processes of animals in fire-prone environments (such as post-fire recovery mechanisms) is critical if we are to understand how species will respond to novel fire regimes or fire management strategies. An important distinction to make is whether post-fire recovery stems from surviving individuals (*in situ* survival) or is driven by immigration (recolonisation) (Banks et al. 2017). For instance, dependence on immigration makes population persistence susceptible to fire size with respect to colonisation capability (Romme et al. 1998). If recovery is *in situ*, there can be major impacts of fire recurrence on genetic diversity (Davies et al. 2016). These mechanisms depend on the life history traits of an organism and may vary depending on the intensity and spatial scale of fires (Hein and Jacob 2015, Mutz et al. 2017). For example, after a severe wildfire, Banks et al. (2017) found that population recovery mechanisms differed between two small mammal species. While agile antechinus recovered through *in situ* survival within the burnt area, bush rat populations followed a pattern of nucleated recovery from topographic refugia. This difference was driven by the impacts of the fire on the specific habitat requirements of these small mammal species. Thus, knowledge of recovery mechanisms can help us to identify the spatial components of fire regimes that are critical to maintaining mammals in the landscape (Clarke 2008, Driscoll et al. 2010).

Fire management is becoming increasingly important as fire regimes change globally (Flannigan et al. 2009, Turner 2010). Changed fire regimes have been connected to species declines in several regions (Gill and Bradstock 1995, Abrahamson and Abrahamson 1996, Templeton et al. 2011), including the broad scale collapse of northern Australia's mammal fauna (Woinarski et al. 2001, 2011). A regime of frequent, extensive, high intensity fire has been observed in northern Australia since the breakdown of traditional burning under Aboriginal custodianship (Russell-Smith et al. 2003, Yates et al. 2008). Traditional burning practices likely resulted in lower intensity fires that were patchily distributed, both temporally and spatially (Vigilante 2001, Legge et al. 2011b). This shift towards extensive late dry season wildfire, and its interaction with other threats, has been implicated in the decline of many small mammal species across this region (Woinarski et al. 2011, Lawes et al. 2015b, Ziembicki et al. 2015).

In northern Australia, manipulative fire experiments and correlative studies have been pivotal in helping us to understand how small mammal populations respond to fire (Andersen et al. 1998, Williams et al. 2003, Legge et al. 2008, Woinarski et al. 2010, Lawes et al. 2015a, Leahy et al. 2016). Many of these studies have shown that small mammals are sensitive to fire in general, potentially suggesting that different modes of recovery may mediate the impact of fire on mammal populations (Romme et al. 1998, Leahy et al. 2016). Despite this, few studies have investigated the mechanisms underlying the post-fire recovery process and how this changes with the size and intensity of fire. Genetic analyses can provide this type of information, by helping us to determine the source of individuals after population perturbance (Peakall and Lindenmayer 2006). This knowledge can then be incorporated into fire management strategies to help facilitate the recovery of small mammals across the landscape.

Here, we use genetic analyses to investigate population recovery in a native Australian rodent, the pale field-rat (*Rattus tunneyi*) following a manipulative fire experiment in the Kimberley region of north-western Australia. We explore how genetic patterns change from before the fire experiment, to one year after fires of differing spatial scales and intensity (with treatments classified as patchy or thorough). In Chapter 4, we found that the pattern of numerical recovery in pale-field rat populations was the

same one year after both patchy and thorough fires. However, differences in the pattern of abundance immediately after fire suggested that the recovery process may differ between these experimental treatments. There was evidence of *in situ* survival in unburnt refuges after patchy fires, but no local survival in sites burnt thoroughly, suggesting that different recovery modes (recolonisation or *in situ* recovery) might operate. This difference is important because it suggests that thorough fires (for example late dry season wildfires, which tend to be extensive) are a threat to population persistence if fire size is large relative to recolonisation capacity (Romme et al. 1998).

In the current study, we test two alternative recovery hypotheses: *in situ* survival and recolonisation (stemming from multiple sources or from a single source). While these mechanisms are not mutually exclusive, genetic patterns are expected to be indicative at the two extremes (Peakall et al. 2006). We develop a set of genetic and demographic predictions (Table 1) to address these hypotheses:

I. *Do patterns of genetic diversity change after fires of differing spatial scales?*
   After patchy fires where post-fire survival was high (Chapter 4), we predict that genetic diversity will be maintained under a model of *in situ* survival (Banks et al. 2017). Alternatively, after extensive thorough fires, we predict that recovery will proceed through recolonisation. Following meta-population extinction-recolonisation models, we predict that within population diversity will increase if recolonisation stems from multiple sources, and decrease if recolonisation comes from a single source (Pannell and Charlesworth 2000).
II. *Do patterns of relatedness change after fires covering differing spatial scales?*
   We compared relatedness estimates before fire, one year after fire and between these two sessions. Under a recovery model of *in situ* survival, we predict that individuals would be more related after fire due to nucleated recovery from unburnt patches, and post-fire individuals will be related to those that were present pre-fire. Alternatively, pre- and post-fire individuals will be unrelated if recovery is driven by recolonisation. We predict that, within sessions, recolonisation from multiple sources will result in lower levels of relatedness one year after fire compared to pre-fire populations, while similar levels of relatedness would suggest recolonisation stemmed from a single source.

Methods

Study species

Once widely distributed across the Australian continent, pale field-rats have suffered a severe range reduction in recent decades and their distribution is now mostly limited to the monsoonal tropics (Braithwaite and Griffiths 1996, Cole and Woinarski 2000, Woinarski et al. 2014). Like many small mammal species in northern Australia, evidence suggests that pale field-rats are vulnerable to predation by feral cats, habitat degradation by introduced herbivores and inappropriate fire regimes (Braithwaite and Griffiths 1996, Legge et al. 2008, 2011a, Woinarski et al. 2010, 2011, 2014, Leahy et al. 2016).

Pale field-rats show strong preferences for riparian habitat and dense grassland in moist, productive environments (Chapter 4; Braithwaite & Griffiths 1996; Braithwaite & Muller 1997; Start *et al.* 2012). They construct extensive, multi-entrance, shallow burrows in sandy soils covering areas up to approximately 20 m^2 (Braithwaite and Griffiths 1996). While population sizes can fluctuate with seasonal conditions, the peak breeding period typically occurs between March – April of each year (Taylor and Calaby 2004). Home range sizes differ between the sexes, with male home range 0.39 ha on average, compared to 0.09 ha in females (Leahy et al. 2016). Generation length in this species is likely 1-2 years (Woinarski et al. 2014).

Study location

This study was carried out at the Mornington Wildlife Sanctuary (17.55°S, 126.17°E), in the central Kimberley region of Western Australia (Fig. 1). Mornington is a former pastoral station that has been managed by the Australian Wildlife Conservancy (AWC) since 2004. This 320,000 ha property has a monsoonal climate, with an average annual rainfall of 750 mm (Bureau of Meteorology) falling mostly in the wet season (December–March). We trapped pale field-rats across an area of the sanctuary that has been destocked of introduced herbivores (primarily cattle) since 2004-2005 (Legge et al. 2011a). Additionally, this area has been part of EcoFire, a collaborative early dry season prescribed burning project, since 2007 (Legge et al. 2011b). A core objective of EcoFire is to reduce extensive, intense fires and increase the amount of long-unburnt habitat.

The fire experiment

We implemented a prescribed burning experiment in 2015, trapping pale field rats across ten sites from February 2015 – July 2016 (Fig. 1; also summarised in Chapter 4). Sites were grouped to include a paired treatment and control (burnt and unburnt) site situated along the same ephemeral watercourse (although one group contained two treatment sites). In total, there were four paired treatment-control groups and one additional unpaired control. Paired sites were between 100 – 1000 m apart. Study sites were situated in open savanna woodland, dominated by tussock and hummock grass communities, with a sparse eucalypt overstorey. Fire return intervals in northern Australia are often short, with fires typically occurring over 2 – 3 year intervals (Yates et al. 2008). Our study sites had not been burnt for at least two years prior to this study.

We applied different fire treatments across five sites in a before-after-control-impact (BACI) design (Fig. 1). These treatments included a patchy or thorough burn (paired with an unburnt control). Patchy fires (implemented across two sites) were typical of early dry season management burns, such as those carried out through the EcoFire project (Legge et al. 2011b). Thorough fires (implemented across three sites) were representative of late dry season wildfires. In patchy treatments, the percentage of the area burnt within a 50m radius of the site was <50%, whereas >50% of the site was burnt in thorough treatments (determined through aerial mapping; see Figure 2a-b for examples and Chapter 4 for further detail).

Trapping protocol and DNA extraction

Pale field-rats were trapped across all ten sites during three trapping sessions, however, because small sample sizes precluded meaningful genetic analyses of individuals in the immediate post-fire period, we focus on the first trapping session (immediately before fire, February – May 2015) and the third trapping session (one year after fire, April – July 2016). Site layout included 100 steel Sherman Type A traps (30 x 10 x 8 cm) arranged in two 1km transect lines approximately 30 – 40 m apart. Each transect was made up of 50 traps spaced at 20m intervals. Trapping was carried out over five nights per site, and traps were baited with rolled oats and peanut butter in the afternoon and checked before sunrise the following morning. Paired sites (within the same group) were trapped within

one week of each other (with the exception of the Group 3 control site, which was trapped 3 weeks after the last treatment site was closed). Processing of captured animals involved species and sex identification, followed by taking a tissue sample from the ear (which was stored in 70% ethanol). Animals were subsequently released at the point of capture. DNA extraction was carried out using proteinase K digestion, protein 'salting out' and ethanol precipitation (Miller et al. 1988).

Demographic study

For the purpose of the present genetic study, we focus on individuals captured within the first and third trapping sessions. However, this research was carried out as part of a broader project, with the demographic outcomes of the fire experiment summarised in Chapter 4. During the demographic component of this research, we also implanted pale field-rats with Trovan ID100 Midi-Chips (Microchips Australia Ltd, Melbourne) for a capture-mark-recapture study. Here, we summarise capture patterns in the first and third trapping sessions, as well as the maximum distances covered by recaptured animals.

DArTseq SNP Genotyping

Single nucleotide polymorphism (SNP) genotyping was carried out by Diversity Arrays Technology (DArT) Pty Ltd. DArTseq is a proprietary reduced representation Next-Generation Sequencing method, which uses restriction enzymes optimised for the target species to achieve complexity reduction (Kilian et al. 2012, Cruz et al. 2013). Pale field-rat extracted DNA (~500ng/sample at a total volume of 10µL) underwent a Zymo purification step (Zymo Research, California, USA) and was digested using the restriction enzyme combination PstI and SphI. Samples were sequenced at approximately 1.5 million reads/sample (or approximately 1.8 million reads/sample with technical replication), on an Illumina Hiseq2500. Read assembly, quality control and SNP calling was carried out through DArTseqTM proprietary analytical pipelines (described in Melville *et al.* 2017) and sequences were BLASTed against the *Rattus norvegicus* reference genome (Rnor6.0; Gibbs *et al.* 2004), to provide a chromosomal position for subsequent filtering on linkage disequilibrium. This resulted in 59,459 bi-allelic SNP loci.

Following DArTseq processing, we filtered SNPs to obtain an informative set of loci for genetic analysis, using a custom R script (R Core Team 2017). In Chapter 3, we

explored the impact of filtering on downstream population genetic analyses and identified key filters that returned the best possible set of high confidence genotypes. Here, we followed the same optimised methods as outlined in Chapter 3. This included removing sex-linked SNPs from the dataset, filtering on <5% missing data, >95% reproducibility (calculated using DArT technical replicates), an average read depth of 10 (for both the reference and SNP) and a minor allele frequency >5%. All loci conformed to Hardy Weinberg Equilibrium in the majority of test populations (tested using the R package HardyWeinberg; Graffelman 2015) and had an observed heterozygosity of <0.6. Finally, SNPs were in approximate linkage equilibrium, with LD filtering carried out in the R package SNPRelate (Zheng et al. 2012) using a correlation threshold 0.2. The final dataset included 3,853 SNP loci for 637 unique individuals.

Statistical analyses

Genetic patterns pre-fire experiment

We calculated common genetic summary statistics across all sites, including observed heterozygosity (H_O), expected heterozygosity (H_E) and the inbreeding coefficient (F). While all SNPs were bi-allelic, we also calculated the number of alleles (N_A), percentage of polymorphic loci ($\%P$) and information index (I) to determine if diversity differed between populations (for example, if some populations were monomorphic for some SNP loci). Summary statistics were calculated using GenAlEx 6.51 (Peakall and Smouse 2006, 2012).

We investigated both individual-level and population-level patterns of genetic structure across pre-fire experiment populations using GenAlEx 6.51 (Peakall and Smouse 2006, 2012). Chapter 3 results suggested that dispersal might be somewhat male-biased in this species. Therefore, genetic analyses were performed separately for females and males. We pooled samples by location (across paired sites within a group) and estimated F_{ST} for all pairwise population comparisons using an analysis of molecular variance (AMOVA) (Excoffier et al. 1992, Peakall et al. 1995). These results were compared to pairwise geographic distances between populations in a Mantel test for matrix correspondence (Smouse et al. 1986, Smouse and Long 1992). This was then repeated for post-fire experiment populations (session 3), to determine how fire might influence

patterns of isolation by distance. Statistical significance was determined using 1000 random permutations, with a significant correlation suggesting a pattern of isolation by distance.

Individual-level patterns of fine-scale genetic structure (tens to thousands of metres) were explored using spatial autocorrelation analysis of multilocus genotypes. This analysis calculates an autocorrelation coefficient, *r*, based on pairwise squared genetic distances that fall within a specified geographic distance class. Thus, *r* describes the genetic similarity between all individuals within a certain geographic threshold (Smouse and Peakall 1999, Peakall et al. 2003, Double et al. 2005, Banks and Peakall 2012). We investigated spatial autocorrelation over several spatial scales; across the entire study landscape (20km with 2km distance classes), within paired sites (2km with 200m distance classes) and within single sites (1km with 100m distance classes). When investigating spatial genetic structure within sites (or within paired sites), we used the 'multiple population' approach, which estimates the combined *r* (r_c) over populations to avoid artificial inflation due to pooling samples from distinct subpopulations (Banks and Peakall 2012).The choice of distance class is a trade-off between the number of pairwise comparisons within the specified distance class (sample size) and the true extent of positive spatial structure (Double et al. 2005, Banks and Peakall 2012). In order to optimise this trade-off, we also calculated r_c for distance classes of increasing size. The distance at which r_c is no longer significantly positive approximates the extent of positive genetic structure (Double et al. 2005). Statistical significance was tested using 1000 bootstrap samples.

Demographic outcomes of the fire experiment

We fitted a generalized linear mixed model (GLMMs) to determine how pale field-rat abundance was influenced by both patchy and thorough fires, using the R package glmmADMB (Skaug et al. 2016). Abundance was our response variable, with fixed effects of treatment (control, patchy, thorough) and session (pre-fire: session 1, and one year post-fire: session 3) and an interaction between these variables. A random effect of group was also included, to account for repeated measurements and spatial location. The model was fitted as a Gaussian GLMM after testing for normality.

How does genetic diversity change after fires covering different spatial scales?

To determine how patterns of genetic diversity are influenced by both patchy and thorough fires, we fitted additional GLMMs based on the summary statistics measured for each site before fire and one year after fire. We tested how *%P*, *I*, H_O, H_E and *F* were effected by patchy and thorough fires. As all SNP loci were bi-allelic, we used *%P* instead of N_A in the model, as both reflect the number of loci that are monomorphic. The response variables represented the mean estimates over all loci, within each site. Models followed the same structure as the demographic GLMM. We also investigated pairwise F_{ST} patterns before fire compared to one year after fire; however, these did not appear to vary and so are not presented here (Appendix S1).

We also investigated how patterns of genetic diversity changed with fire treatment using QDiversity analysis in GenAlEx 6.51 (Peakall and Smouse 2006, 2012). This new diversity analysis is based on Rao's Quadratic Entropy and translated into scaled diversity analogues [0,1], as outlined in Smouse, Banks, & Peakall (2017). Unlike AMOVA and similar F_{ST} related methods, it allows patterns of genetic diversity to be quantified and statistically evaluated both within and among strata of nested hierarchical levels. We tested for genetic heterogeneity between sessions (before and one year after fire) within control, patchy and thorough sites. We statistically evaluated this heterogeneity using 1000 random permutations of alleles among sites and sessions to test for significant heterogeneity across the landscape (δ') and to test if heterogeneity differed between sessions (from before fire to one year after fire, β'). Bartlett's test of homogeneity was used to statistically test whether diversity (α') within patchy, thorough and control sites was homogeneous across sessions, using 1000 random permutations.

How does relatedness change after fires covering different spatial scales?

We explored how patterns of relatedness changed from before the fire experiment to one year after patchy and thorough fires were implemented, as compared to the unburnt controls. The use of thousands of SNPs can provide reliable and detailed estimates of relatedness and there are a number of approaches for estimating these patterns (Attard et al. 2018). Here, we used a maximum likelihood parentage analysis and spatial autocorrelation to explore fine-scale patterns of relatedness between individuals. We compare these patterns to the distribution of maximum distances travelled between

traps by recaptured pale field-rats. We used within and between session movements over the entire study (including the trapping session immediately after fire).

We used the R package SEQUOIA (Huisman 2017) to assign relatives across all individuals in our study. SEQUOIA is a maximum likelihood method that combines genetic and demographic information. In this case, the age and sex of pale field-rats were included as priors in the analysis. This method then compares the likelihood of all possible relationships between pairwise comparisons of individuals to the alternative of being unrelated, using a heuristic hill-climbing algorithm. Simulation testing using realistic genotyping error rates and amounts of missing data found that this method was highly accurate, even when using as few as 100 independent SNP loci (Huisman 2017). We assigned first-order (parent-offspring and full siblings), second-order (half-siblings, grandparent-grand offspring and full aunt/uncle-niece/nephew) and third-order (great grandparent- great grandoffspring, half aunt/uncle-niece/nephew and full cousins) relatives to individuals across all sessions and sites. However, because it was not possible to correctly identify the age of individuals that were first caught as adults, we were unable to determine specific relationships within each relatedness category. The resulting assignments were used to calculate the proportion of individuals with a relative within each site and between trapping sessions (from before the fire experiment to one year after patchy and thorough fires), for each category of relatedness (first-, second- or third order).

Spatial autocorrelation analysis was used to test whether fine-scale patterns of spatial genetic structure (r_c) changed from before the fire experiment, to one year after patchy and thorough fires (compared to controls). While distance-based spatial autocorrelation analysis is generic and not exclusive to genetic data, when using genetic data estimates of *r* are strongly correlated with relatedness (Smouse et al. 2008). We estimated r_c separately across patchy, thorough and control sites for all individuals within a distance class of 100m, as this was the spatial scale over which the strongest genetic structure was detected (see results). This was performed within each session (before fire and one year after fire), as well as between sessions (for pairwise comparisons of individuals within 100m of each other). This enabled us to investigate whether fire had an impact on the spatial distribution of genotypes, and whether individuals present in

session 3 were genetically similar to those that were present in session 1 (and if this changed across the different fire treatments).

Results

Genetic patterns pre-fire experiment

Summary statistics

We captured 324 pale field-rats during session 1 (control= 194, patchy= 36, thorough = 94), compared to 313 in session 3, one year after the fire experiment (control= 194, patchy= 45, thorough = 74; Table 2). Genetic diversity estimates were broadly similar across sites, with a mean observed heterozygosity of 0.23 ±0.000 (0.23 – 0.24), a mean expected heterozygosity of 0.26 ±0.001 (0.25 – 0.27) and a mean inbreeding coefficient of 0.09 ±0.007 (0.06 – 0.12). The mean N_A was 1.95 ±0.013 (1.89 – 2), with 95.38% of loci polymorphic on average. The mean information index across loci was 0.41 ±0.003 (0.4 – 0.42). All summary statistics are summarised in Table 2.

Population-level genetic structure

Pairwise F_{ST} values indicated low, but significant genetic structure was present across the pre-fire experiment landscape (Appendix S1). Significant population differentiation was detected between all grouped sites, with pairwise F_{ST} ranging from 0.007 – 0.013 (Fig. 3; Appendix S1). Mantel tests revealed a strong, significant positive correlation between genetic and geographic distance matrices for females in session 1 and session 3 (session 1: Rxy= 0.813, p= 0.032; session 3: Rxy= 0.722, p= 0.008). However, this pattern was weaker in males (session 1: Rxy= 0.496, p= 0.078; session 3: Rxy= 0.657, p= 0.034).

Individual-level genetic structure

The maximum distance travelled by recaptured animals, within and between trapping sessions, is summarised in Figure 4. Within trapping sessions, recaptured animals moved an average distance of 33 ± 4.63 m (maximum= 522.23 m), with males moving further than females (52.78 ± 11.09 m compared to 20.71 ± 11.09 m, respectively). Between trapping sessions, recaptured animals moved an average distance of 108 ± 54.58 m (maximum = 1882 m).

Spatial autocorrelation analysis of the total study landscape (0 – 20km) showed significant spatial genetic structure over the within group scale (<2km) for both females (r= 0.012) and males (r= 0.009; Fig. 4). Within grouped sites (0 – 2km), significant positive genetic structure was detected within the first distance class of 200m (females: r_c= 0.020, males: r_c= 0.016; Fig. 4). This structure was likely driven by a non-random distribution of genotypes within the first 100m, as demonstrated by the within site correlogram (0 – 1km), which showed significant spatial genetic structure in the first distance class (females: r_c= 0.041, males: r_c= 0.025; Fig. 4). This fine-scale structure rapidly decreased to zero by 200 – 300m. This was also supported by a multiple distance class analysis, where the strongest genetic structure was revealed using a 100m distance class, however positive spatial genetic structure was detectable until 700 – 900m (for females and males respectively; Fig. 4). While females consistently showed greater fine-scale genetic structure than males, this difference was not significant at any spatial scale (female and male 95% bootstrap confidence intervals (CIs) overlapped). Removing juvenile pale-field rats from the analysis resulted in a decrease in fine-scale spatial genetic structure (r_c) from 0.025 to 0.015 for males at the 100m distance class. It also resulted in larger confidence intervals around all estimates, although it did not impact the significance of fine-scale genetic patterns (Appendix S2).

How does genetic diversity change after fires covering different spatial scales?

Thorough fires had a significant positive effect on the percentage of polymorphic loci (*%P*) in session 3, one year after the fire experiment (p<0.05; Table 3). Furthermore, the information index (*I*) also increased one year after thorough fires, although this effect was not statistically significant (p=0.088; Table 3). While patchy sites had fewer polymorphic loci before the fires were implemented (p<0.05), there was no significant effect of patchy fires on either *%P* or *I* one year later. In control sites, *%P* and *I* were not influenced by session, meaning that these diversity metrics were stable over time. There was no significant impact of treatment (control, patchy fire or thorough fire) or session across the remaining diversity metrics (H_O, H_E and *F*; Appendix S3).

QDiversity analysis revealed low, but significant genetic differentiation among sites (δ'= 0.007, p<0.01; Table 4). However, genetic heterogeneity did not change significantly

within sites, between sessions (β'= 0.008, p= 0.108; Table 4). This was also true when comparing within session genetic heterogeneity separately across each site (mean α'= 0.282 – 0.288, p= 0.681 – 0.975; Appendix S4), suggesting that both thorough and patchy fires did not significantly alter patterns of genetic diversity.

How does relatedness change after fires covering different spatial scales?

Overall, the average proportion of individuals assigned a relative within the same site (within approximately <1km) did not change from before the fire experiment (session 1: control= 69±14%; patchy= 20±20%; thorough= 50±25%) to one year after fires were implemented (session 3: control= 75±15%, patchy= 56±36%, thorough= 62±1%), across both treatment and control sites (although, the proportion of each relatedness category did vary; Fig. 5). However, the proportion of related individuals between sessions (session 1 – session 3) decreased in treatment sites compared to control sites from an average of 35% ± 9% in controls, to 10% ± 10% in patchy sites and 3% ± 2% in thorough sites (control: first-order= 4±2%, second-order= 10±3%, third order= 21±5%; patchy: first-order= 0%; second-order= 4±4%; third order= 6±6%; thorough: first-order= 0%; second-order= 1±1%; third order= 3±3%; Fig. 5).

While similar numbers of animals were captured in both trapping sessions, only six of the animals captured in session 3 were recaptures from session 1 and all of these were from control sites (Fig. 6). Genetic spatial autocorrelation analysis revealed some differences in relatedness between control and treatment sites. Across both control and treatment sites, similar levels of positive spatial genetic structure were detected in session 1 and session 3 (control: r_c= 0.03 vs. 0.03, patchy: 0.02 vs. 0.03, thorough: r_c= 0.04 vs. 0.03; Fig. 6). This structure was significant in all cases, with the exception of patchy sites in session 1, where small sample sizes resulted in 95% bootstrap CIs overlapping zero. While significant positive genetic structure was detected between sessions within control sites (control: r_c= 0.01), 95% bootstrap CIs overlapped zero in patchy sites (patchy: r_c= 0), and significant negative structure was detected in thorough sites (thorough: r_c= -0.01). These results were consistent when juveniles were removed from the analysis, although lower sample size resulted in larger confidence intervals and thus some patterns were no longer significant (Appendix S5).

Interestingly, when separate analyses were run for each sex, different patterns of fine-scale genetic structure were detected after patchy versus thorough fires (Fig. 6). Pairwise comparisons of pre-fire individuals to those present one year after patchy fires revealed negative structure in males, compared to positive structure in females (although in both cases 95% CIs overlapped zero; female r_c= 0.03, male r_c= 0.01). In session 3, one year after patchy fires, this pattern was maintained, with female fine-scale genetic structure significantly higher than that found in males (female r_c= 0.06, male r_c= -0.01; Fig. 6). Conversely, pairwise comparisons of pre-fire individuals to those present one year after thorough fires revealed no structure in males, compared to significant negative structure in females (female r_c= -0.01, male r_c= 0). One year after thorough fires (session 3), patterns of fine-scale genetic structure were similar to those detected before fire, though female structure had decreased (session 1: female r_c= 0.05, male r_c= -0.02; session 3: female r_c= 0.03, male r_c= -0.02; Fig. 6). In control sites, fine-scale genetic structure remained consistent both within and between sessions (session 1: female r_c= 0.03, male r_c= 0.02; session 3: female r_c= 0.03, male r_c= 0.02; session 1 – 3: female r_c= 0.02, male r_c= 0.01; Fig. 6).

Discussion

In northern Australia, small mammal populations are currently collapsing (Woinarski et al. 2011). In fact, a large percentage (24% – 53%) of mammal species have been classified as threatened within all of the most specious taxonomic groups in this region (Ziembicki et al. 2015). Of the native rodents in northern Australia, 30% are extinct, threatened or near threatened. Extensive, spatially homogeneous fires are implicated in many of these declines. Extensive wildfire has had a demonstrated negative effect on the survival of small mammal species (Pardon et al. 2003, Legge et al. 2008). Additionally, Lawes et al. (2015b) found that fire extent was the best predictor of mammal declines in Kakadu conservation reserve.

Here, we investigate how pale field-rats recover after fire events, and how fire characteristics (extent and patchiness) might influence the recovery process. In Chapter 4, we determined the spatial distribution and abundance of survivors after fire, and identified the starting point for population recovery. In the present study, we combine

demographic and genetic evidence to help elucidate the recovery process in this vulnerable native rodent. Our findings support the hypothesis that recovery proceeds differently depending on the spatial extent and patchiness of fires. Furthermore, our genetic findings reveal insight into population processes in this disturbance-prone landscape.

Genetic patterns pre-fire experiment

Northern Australia is a landscape defined by disturbance, dominated by fire in the dry season and flooding in the wet season (Woinarski et al. 2005). We investigated the genetic characteristics of pale field-rat populations prior to our fire experiment, to determine the base-line genetic patterns shaped, in part, by this disturbance regime. Our findings revealed low, but significant estimates of F_{ST} across our study landscape (<20 km). Despite low population genetic structure, a significant correlation between genetic and geographic distance was detected for both sexes (males in session 3 only), indicative of isolation by distance. Significant local spatial genetic structure was also detected over a 100 m scale, meaning that individuals within 100 m of each other were more related than those further apart (consistent with mark-recapture results). Female structure was slightly higher than that found for males, although we did not detect a significant signature of male-biased dispersal. While these patterns are indicative of local restrictions to dispersal, low estimates of F_{ST} suggest that populations are connected, such that gene flow occurs between populations.

Similarly, Peakall & Lindenmayer (2006) also found genetic evidence for restricted dispersal in another species of native rodent, the Australian bush rat. They suggested that bush rat movement may be restricted to watercourses. In the monsoonal tropics, non-riparian zones may become inhospitable to pale field-rats as the dry season progresses. Braithwaite & Griffiths (1996) suggested that pale field-rats have relatively poor dispersal capabilities compared to other *Rattus* species and population expansion occurs from riparian zones. Furthermore, in Chapter 4, we found strong habitat preferences for vegetation types typically situated along creek lines and rivers. While pale field-rats are found in black soil plains, away from moist habitat, their numbers are at much lower densities in these areas (*A. James, personal comm.).* This, coupled with evidence for some

per generational restricted gene flow, suggests that pale field-rat source populations (and potentially dispersal pathways) are primarily restricted to creek lines, rivers and other moist, productive habitats.

Nevertheless, we did find evidence for gene flow across the scale of our study (~20 km). In addition, isolation by distance patterns in males varied between trapping sessions. While this could have been a direct impact of our fire experiment, we did not observe a consistent effect of the different fire treatments on the magnitude of F_{ST}. The fact that F_{ST} estimates were low and variable across sites and sessions may instead reflect pale field-rat life history characteristics, shaped by the unpredictable nature of savanna ecosystems. Regular disturbance followed by regeneration means that the habitat preferred by pale field-rats is dynamic in space and time (Bowman et al. 1988, Russell-Smith et al. 1998). Thus, dispersal may occur in response to disturbance and environmental variability, while restricted habitat requirements result in the build-up of local genetic structure. In such stochastic ecosystems, theoretical models predict the evolution of high dispersal rates (Van Valen 1971, Friedenberg 2003, Duputié and Massol 2013). However, this is balanced against landscape related costs of dispersing (McPeek and Holt 1992, Travis and Dytham 1999). Thus, population-level gene flow coupled with the presence of local genetic structure may represent the interplay between these two forces.

Demographic outcomes of the fire experiment

We made a series of demographic and genetic predictions about the outcomes of patchy versus thorough fires, resulting in different recovery hypotheses (Table 1). We predicted that pale field-rat abundance would initially decline after patchy fires (relative to control sites), with rapid recovery facilitated by residual surviving animals within unburnt patches. Thus, we predicted that recovery after patchy fires would follow a model of *in situ* survival. Our demographic results support these predictions, with both pale field-rat abundance and recapture rate negatively correlated with increasing fire extent immediately after fires and surviving pale field-rats distributed within unburnt patches (Chapter 4). Abundance one year after patchy fires had recovered to pre-fire levels.

Alternatively, we predicted complete mortality immediately after thorough fires, followed by slow recovery (lower abundance one year later). Our recovery hypothesis was therefore recolonisation from outside of the burnt area. Indeed, total captures decreased by 95% immediately after thorough fires, with no pre-fire animals recaptured (Chapter 4). However, contrary to our prediction of slow recovery, pale field-rat abundance completely recovered one year after thorough fires. In Chapter 4, we suggested that rapid recovery was due to the scale of our fire experiment, with unburnt areas outside of the treatment site within approximately 100 m – 1km of the site perimeter. However, strong habitat preferences suggest that source populations (from which recolonisation stemmed) may have been restricted to creek lines.

Do patterns of genetic diversity change after fires of differing spatial scales?

Computer simulations have shown that disturbance has a relatively minor impact on genetic diversity when *in situ* survival is high or disturbance size is small relative to the dispersal capabilities of an organism (Davies et al. 2016). Thus, we predicted that one year after patchy fires were implemented, measures of genetic diversity (heterozygosity, α' diversity and β' diversity) would be similar to pre-fire estimates (genetic predictions also found in Table 1). Our results followed these predictions. We found no effect of patchy fires on heterozygosity, α' diversity, β' diversity, the percentage of polymorphic loci or the information index of loci. This was likely due to high pale field-rat survival within patchily burnt sites.

Alternatively, theoretical work on extinction-recolonisation dynamics in metapopulations predicts that neutral genetic diversity is sensitive to the source of colonists (Pannell and Charlesworth 2000). When migration is high and from a number of source populations, genetic diversity in the focal population should increase. However, the opposite is true if colonisation stems from a single source (Slatkin 1977, Pannell and Charlesworth 2000). Thus, we predicted that a recovery model of recolonisation after thorough fires would result in lowered genetic diversity (heterozygosity, α' diversity and β' diversity) if recolonisation came from a single source, compared to increased diversity if colonists came from multiple sources. In fact, neither of these hypotheses were true, with measures of genetic diversity stable between the pre- and post-fire populations.

However, we did detect an increase in the number of polymorphic loci and the information content of loci one year after thorough fire, providing evidence for recolonisation. Similarly, a study on banner-tailed kangaroo rats found that migration likely explained the arrival of new alleles in the population after a recent bottleneck (Busch et al. 2009).

Do patterns of relatedness change after fires covering differing spatial scales?

We predicted that *in situ* survival after patchy fires would result in high relatedness between individuals caught before and one year after fire, due to nucleated recovery from fewer family groups. However, we found lower levels of relatedness (genetic spatial autocorrelation) in patchily burnt sites compared to controls, although a number of second- and third-order relatives were assigned between sessions. While lower than in control sites, genetic spatial autocorrelation was significantly higher in patchily burnt sites than in those that underwent thorough fires (Fig. 6). Individuals were significantly unrelated between sessions (over a 100 m scale) in thoroughly burnt sites, suggesting complete population turnover. However, fine-scale spatial genetic structure was re-established one year later. Thus, individuals within the post-fire population were genetically similar over a 100 m scale. Our hypothesis stated that this pattern is likely to occur when immigrants come from a single source. Pale field-rat preferred habitat is fairly restricted in this landscape, so it is likely that the number of local sources was low. Potentially, the need for specific (and limited) habitat for establishing a territory could result in 'collective dispersal', or correlated dispersal paths between individuals, resulting in high relatedness of colonists (Yearsley et al. 2013).

Interestingly, these patterns changed when analyses were split by sex. After patchy fires, female spatial genetic structure increased both between sessions and one year after the patchy fires (relative to controls and pre-fire levels; Fig. 6). Conversely, after thorough fires, significant negative spatial genetic structure was detected between sessions in females (proximate individuals were genetically different). However, one year after thorough fires, female structure was similar to pre-fire levels. In all cases (controls, patchy fires and thorough fires), spatial genetic structure was not present in males.

This suggests that fire extent can have an impact on the recovery mechanisms of female pale field-rats. While male dispersal may have remained relatively constant regardless of disturbance, female dispersal may vary in relation to habitat availability. After patchy fires, unoccupied habitat would have been nearby (once the vegetation had recovered), meaning that new female recruits could settle close to their natal territory (increasing philopatry). When compared to male dispersal (often associated with inbreeding avoidance, kin competition or local mate competition; Lawson Handley & Perrin, 2007), this would result in a strong genetic signal of male-biased dispersal. Conversely, females may switch to colonisation behaviour after thorough fires (once the vegetation recovers) to take advantage of the availability of these new resources and territories. This might explain why a signal of male-biased dispersal was not detected before the fire experiment or in control sites, as two dispersal strategies are potentially occurring across the landscape, perhaps diluting the signals of sex-bias that are more evident in other Australian small mammal species (Cockburn et al. 1985, Peakall et al. 2003, Banks and Peakall 2012).

Our results provide strong evidence that females exhibit two dispersal strategies (also known as dispersal polymorphism), likely as a consequence of the extent of our experimental fires and low survival in thoroughly burnt sites. Individual-based modelling has shown that both dispersal distances and dispersal probabilities are likely to increase with increasing habitat availability, particularly when available habitat is continuously distributed (Bonte et al. 2010). Dispersal polymorphism has been discovered in a number of species, often in response to demographic (population density dependence) or environmental processes (resource availability, range expansions, disturbance; Banks et al., 2017; Hovestadt, Mitesser, & Poethke, 2014; Simmons & Thomas, 2004; also reviewed in Appendix S1 of Chapter 2).

Combining genetic analyses and demographic evidence: what have we learned?

Our demographic findings immediately after the fire-experiment (Chapter 4) provided us with strong clues about the starting point for population recovery. Furthermore, the abundance and distribution of individuals before and after fire allowed us to make predictions about the post-fire recovery process and the habitat preferences in this

species. When combined with genetic evidence, our results suggest that *in situ* survival drives population recovery after patchy fires, compared to recolonisation from limited source populations after thorough fires. Furthermore, these patterns appear to be driven by female dispersal polymorphism, with dispersal potentially increasing with fire extent and patchiness. Our capacity to detect this evidence for the operation of two different modes of recovery was unexpected, given the scale of this experiment. The fact that we were able to find strong evidence (with congruent demographic and genetic patterns) over such a fine scale suggests that these are important mechanisms in pale field-rat populations.

Critically, the detailed local patterns revealed here allow us to make predictions about how post-fire recovery might occur after extensive fire scenarios (Table 1). Small mammals appear to be sensitive to fire in general, with evidence for low survival after extensive, homogeneous fire (Pardon et al. 2003, Legge et al. 2008, Lawes et al. 2015b). Thus, recolonisation is likely the main recovery mechanism following these types of fires. The recovery process would likely be slow following large disturbances and exacerbated by restricted dispersal and strong habitat requirements for population establishment. This would limit opportunities for population recovery. Furthermore, the presence of suitable source populations is particularly important in systems that rely on recolonisation for population recovery (Mutz et al. 2017).

In populations of vulnerable mammals, there may be a tipping point, where once a threshold fire size is reached, populations are vulnerable to local extinction. Frequent, extensive, unmanaged fires in northern Australia, in combination with other key threats, may be pushing small mammal populations over this tipping point. Thus, fire management strategies that support survival and maintain suitable recolonisation sources (and connectivity) will be critical for facilitating post-fire recovery in many small mammal populations.

Tables and Figures

Table 1. The demographic and genetic patterns predicted to be associated with different post-fire recovery mechanisms at the site level. Symbols denote patterns supported by our findings (✓), not found in our study (✗), and somewhat supported by our data/or that we were unable to clarify (~). Hypotheses for extensive thorough fires are also presented (although not tested in this study).

Predictions	Hypothesis	Control	Patchy	Thorough Single source	Thorough Multiple sources	Thorough Extensive
Demographic	**Abundance immediately after fire**	Increase due to breeding season ✓	Slight decline (some survival, some mortality, buffered by new recruits) ✓	No survivors ✓	No survivors ✓	*No survivors*
	Recaptures	High recapture rate ✓	Fewer recaptures than controls ✓	No recaptures ✓	No recaptures ✓	*No recaptures*
	Dispersal	Male-biased dispersal ~	Male-biased dispersal ~	No sex bias ~	No sex bias ~	*Local extinctions*
	Recovery rate	No difference in abundance between trapping sessions ✓	Rapid recovery ✓	Slow recovery ✗	Faster recovery than single source ~	*Local extinctions*
	Recovery hypothesis	*Stable*	*Stemming from within the site (unburnt patches): in situ survival*	*Recolonisation from source populations restricted to creek lines*	*Recolonisation from all edges of fire scar*	*Recolonisation if suitable source populations present, otherwise local extinction*

*continued over page

Predictions	Hypothesis	Control	Patchy	Thorough Single source	Thorough Multiple sources	Thorough Extensive
Genetic	**Diversity (heterozygosity, α′ and β')**	Stable across sessions ✓	Stable across sessions ✓	Lower diversity ✗	Higher diversity ✗	*Reduction in diversity due to bottleneck effects*
	Polymorphic loci	Stable across sessions ✓	Stable across sessions ✓	Dependent on source population	Higher polymorphism ✓	*Dependent on source populations*
	Relatedness	Stable across sessions, high between sessions ✓	Stable across sessions, higher between sessions compared to controls ~	High relatedness in both sessions, no relatedness between sessions ✓	Lower relatedness one year after fire, no relatedness between sessions ✗	*Potential founder effect*
	Fine-scale genetic structure	Positive spatial genetic structure within sites for both sexes	Increased spatial genetic structure in females compared to controls, similar patterns to controls in males ✓	Fine-scale spatial genetic structure similar between females and males ✓	Fine-scale spatial genetic structure similar between females and males ✓	*No fine-scale genetic structure*

Table 2. Summary statistics across ten sites where pale field-rats were trapped, before the fire experiment (session 1) and one year after fire (session 3). Number of samples (*N*), number of alleles (N_A), the information index (*I*), the percentage of polymorphic loci (*%P*), observed heterozygosity (H_O), expected heterozygosity (H_E) and inbreeding coefficient (*F*) are presented.

Session	Site	Group	Treatment	*N*	N_A	*I*	*%P*	H_O	H_E	*F*
Pre-fire	RS08	1	Control	39	1.99 ±0.001	0.42 ±0.002	99.32	0.23 ±0.002	0.27 ±0.002	0.12 ±0.003
	RS09	1	Patchy	27	1.98 ±0.002	0.42 ±0.003	97.95	0.23 ±0.002	0.26 ±0.002	0.11 ±0.004
	RS10	2	Control	24	1.96 ±0.003	0.41 ±0.003	96.24	0.23 ±0.002	0.26 ±0.002	0.09 ±0.004
	RS11	2	Patchy	9	1.87 ±0.005	0.40 ±0.003	87.10	0.23 ±0.002	0.26 ±0.002	0.06 ±0.005
	RS12	3	Thorough	14	1.94 ±0.003	0.41 ±0.003	93.64	0.23 ±0.002	0.26 ±0.002	0.08 ±0.004
	RS13	3	Thorough	13	1.89 ±0.004	0.40 ±0.003	89.46	0.24 ±0.002	0.25 ±0.002	0.06 ±0.005
	RS14	4	Control	22	1.97 ±0.002	0.42 ±0.003	97.09	0.24 ±0.002	0.27 ±0.002	0.09 ±0.004
	RS15	4	Thorough	67	1.99 ±0.001	0.42 ±0.002	99.58	0.23 ±0.002	0.27 ±0.002	0.12 ±0.003
	RS16	5	Control	94	2.00 ±0.000	0.43 ±0.002	99.97	0.24 ±0.002	0.27 ±0.002	0.12 ±0.002
	RS17	3	Control	15	1.93 ±0.003	0.41 ±0.003	93.49	0.24 ±0.002	0.26 ±0.002	0.08 ±0.004
	Mean	-	-	32.4 ± 8.7	1.95 ±0.013	0.41 ±0.003	95.38 ±1.39	0.23 ±0.000	0.26 ±0.001	0.09 ±0.007
One year post-fire	RS08	1	Control	57	1.99 ±0.001	0.43 ±0.002	99.58	0.24 ±0.002	0.27 ±0.002	0.11 ±0.003
	RS09	1	Patchy	35	1.99 ±0.001	0.42 ±0.002	99.22	0.23 ±0.002	0.27 ±0.002	0.11 ±0.003
	RS10	2	Control	12	1.88 ±0.005	0.40 ±0.003	88.42	0.23 ±0.002	0.26 ±0.002	0.08 ±0.005
	RS11	2	Patchy	10	1.88 ±0.005	0.40 ±0.003	87.65	0.24 ±0.002	0.26 ±0.002	0.05 ±0.005
	RS12	3	Thorough	27	1.97 ±0.002	0.41 ±0.003	97.53	0.23 ±0.002	0.26 ±0.002	0.09 ±0.003
	RS13	3	Thorough	25	1.98 ±0.002	0.42 ±0.003	97.85	0.24 ±0.002	0.27 ±0.002	0.09 ±0.003
	RS14	4	Control	48	1.99 ±0.001	0.42 ±0.002	99.51	0.23 ±0.002	0.27 ±0.002	0.12 ±0.003
	RS15	4	Thorough	22	1.96 ±0.002	0.42 ±0.003	96.50	0.24 ±0.002	0.27 ±0.002	0.09 ±0.004
	RS16	5	Control	66	2.00 ±0.000	0.43 ±0.002	99.84	0.24 ±0.002	0.27 ±0.002	0.12 ±0.002
	RS17	3	Control	11	1.90 ±0.004	0.40 ±0.003	90.01	0.23 ±0.002	0.26 ±0.002	0.07 ±0.005
	Mean	-	-	31.3 ± 6.3	1.96 ±0.015	0.41 ±0.003	95.61 ±1.56	0.23 ±0.000	0.26 ±0.001	0.09 ±0.007

Table 3. Model summaries for GLMMs investigating the effect of session (S1: pre-fire experiment, S3: one year post-fire experiment) and treatment (control, patchy, thorough) on pale-field rat abundance, as well as the percentage of polymorphic loci (*%P*) and the information index of loci (*I*) across pale field-rat populations.

Response	Variable	Estimate	Std. Error	Z value	p
Pale field-rat abundance	Control (Intercept)	-2.701	0.383	-7.051	**<0.0001**
	Patchy	-0.692	0.414	-1.672	0.095
	Thorough	0.264	0.364	0.725	0.469
	S3	-0.001	0.273	-0.002	0.998
	Patchy x S3	-0.047	0.539	-0.088	0.930
	Thorough x S3	0.124	0.463	0.267	0.790
	Random term	Variance	Standard Deviation		
	Group	0.547	0.7396		
%P	Control (Intercept)	97.223	1.887	51.531	**<0.0001**
	Patchy	-4.326	2.138	-2.024	**<0.05**
	Thorough	-1.190	1.936	-0.615	0.539
	S3	-1.749	1.490	-1.174	0.240
	Patchy x S3	2.658	2.788	0.953	0.340
	Thorough x S3	4.812	2.434	1.977	**<0.05**
	Random term	Variance	Standard Deviation		
	Group	12.24	3.499		
I	Control (Intercept)	0.420	0.005	84.996	**<0.0001**
	Patchy	-0.008	0.006	-1.367	0.171
	Thorough	-0.005	0.006	-0.883	0.377
	S3	-0.004	0.004	-0.924	0.355
	Patchy x S3	0.005	0.008	0.682	0.495
	Thorough x S3	0.012	0.007	1.705	0.088
	Random term	Variance	Standard Deviation		
	Group	7.670E-05	8.758E-03		

Table 4. Raw, translated and scaled [0,1] diversity values for pale field rat treatment and control sites. Sites represent trapping locations and treatments and sessions represent trapping periods (before the fire experiment and one year after fire). Statistical significance was evaluated with 1000 random permutations of alleles among sites and sessions.

Strata	Q	Q_{max}	Raw Diversity		Scaled-Diversity		p
Total	0.289	0.999	γ	1.407	γ'	0.289	-
Within sites	0.285	0.993	σ	1.398	σ'	0.287	-
Among sites	0.006	0.883	δ	1.006	δ'	0.007	0.002
Within sessions	0.282	0.988	α	1.393	α'	0.286	-
Among sessions	0.004	0.453	β	1.004	β'	0.008	0.108
Within individuals	0.117	0.500	ω	1.132	ω'	0.233	-
Among individuals	0.187	0.975	ε	1.231	ε'	0.192	0.001

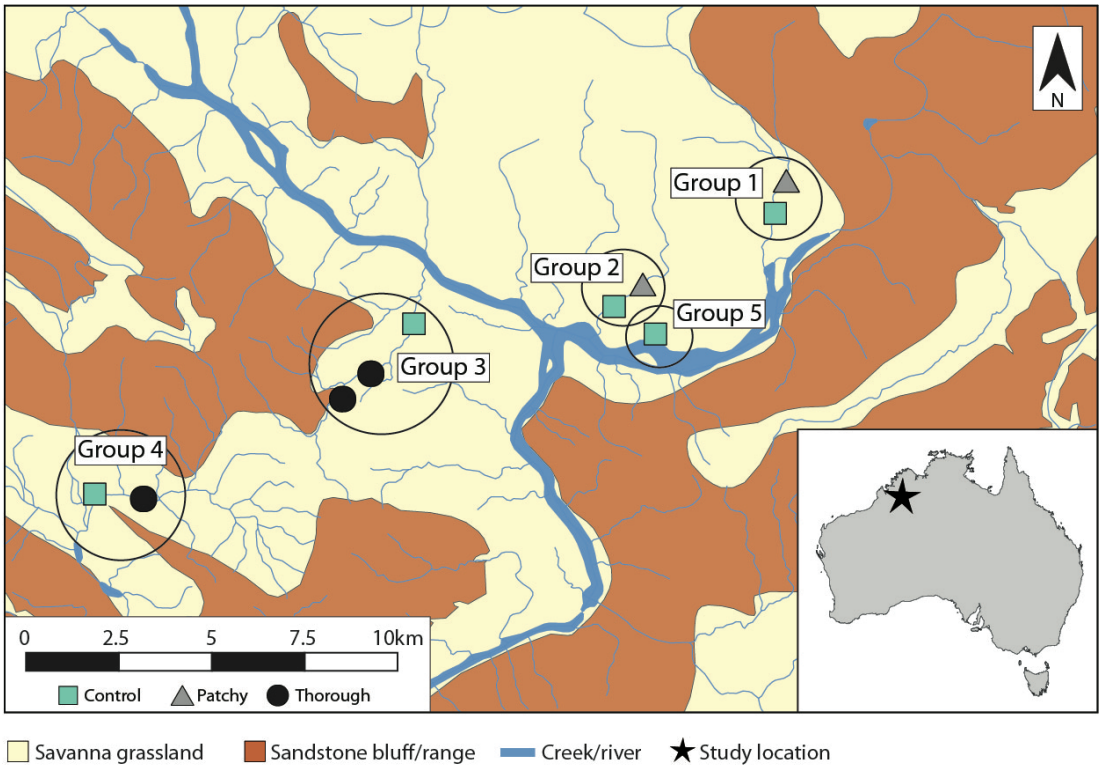

Figure 1. The Mornington wildlife sanctuary. Map of study location displaying the spatial arrangement of sites within groups. All images were edited using Adobe Illustrator CC2014.

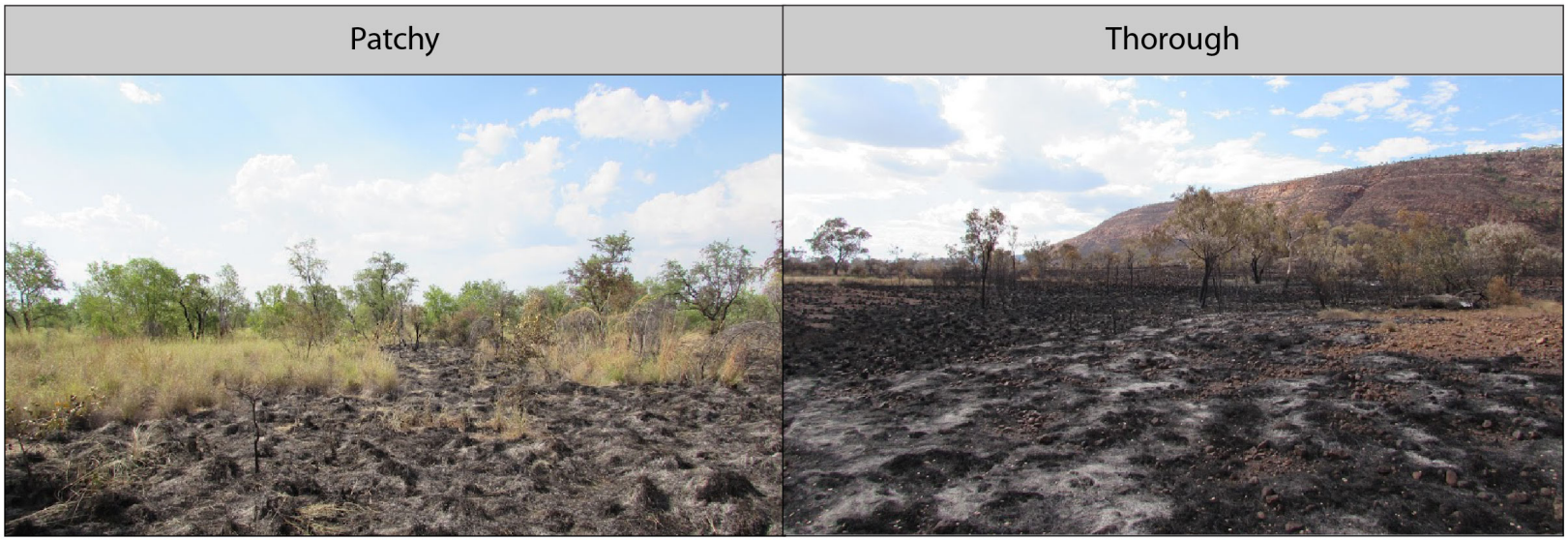

Figure 2a. Examples of patchy and thorough fires from ground-level photographs.

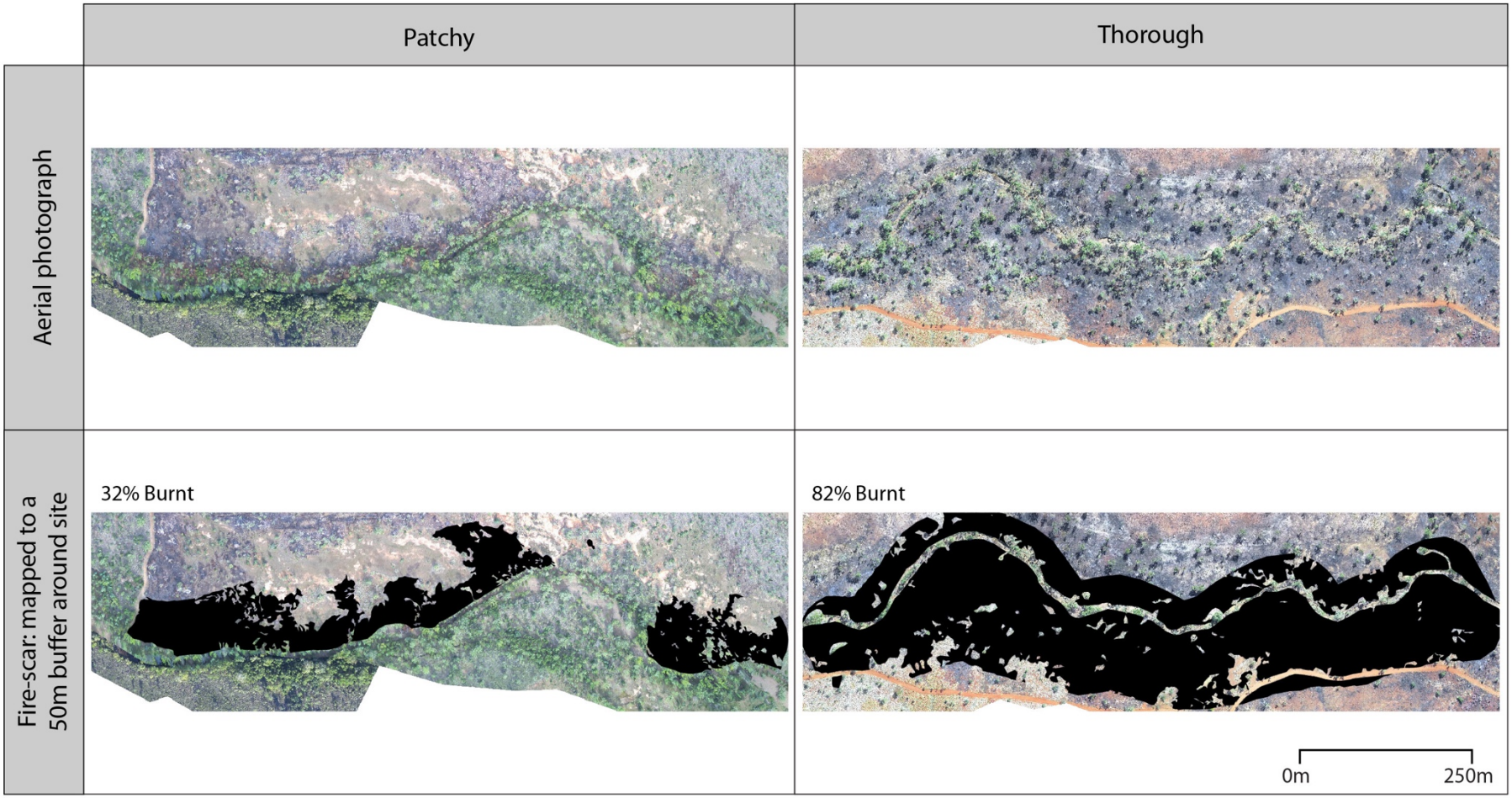

Figure 2b. Examples of patchy and thorough fires from aerial photographs, and the mapped fire scars used to caluclate the percentage of the site that was burnt within a 50m buffer zone of the trap lines.

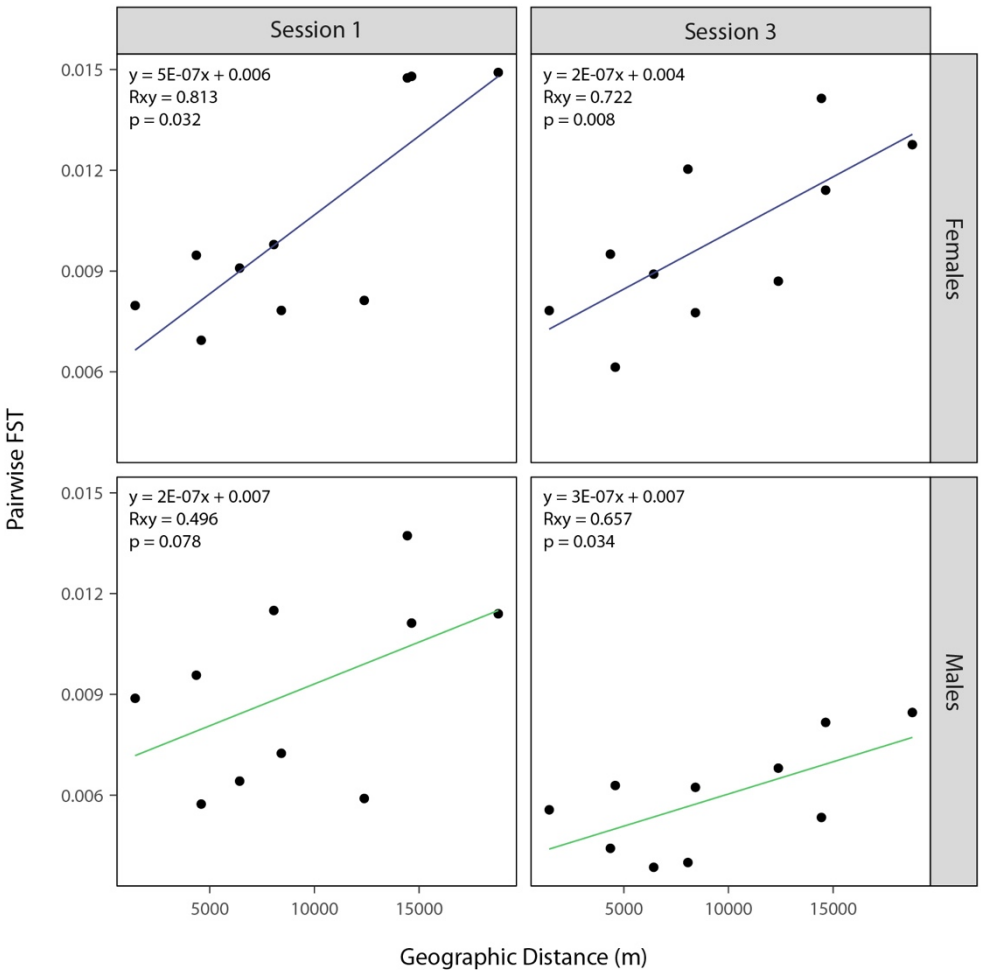

Figure 3. Female and male Mantel tests for matrix correspondence between pairwise F_{ST} values (between site groups) and geographic distances, before the fire experiment was implemented (session 1) and one year later (session 3). P values were determined with 1000 random permutations. Figures were created using the R package ggplot2 (Wickham 2009).

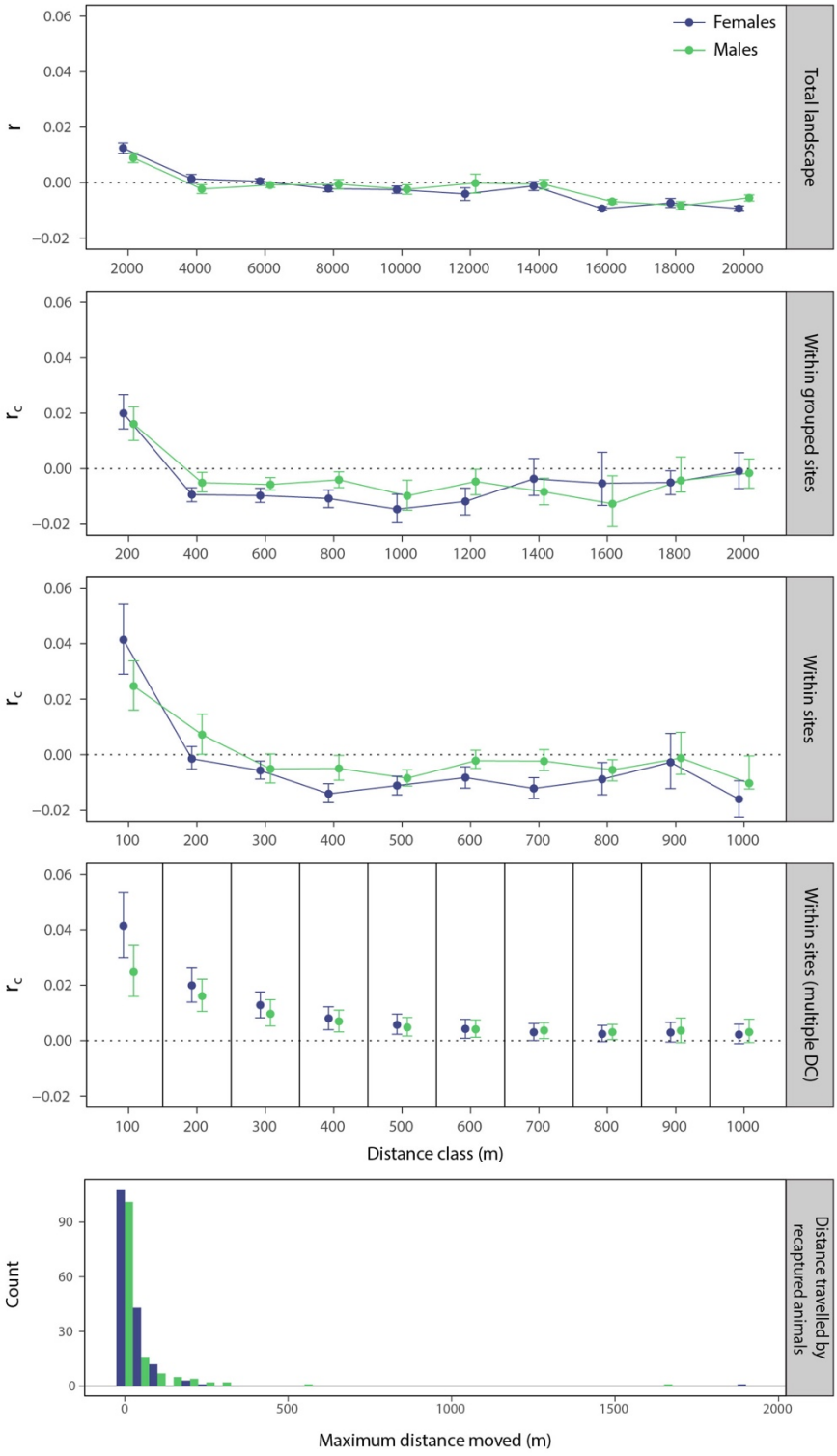

Figure 4. Panels 1-3 show correlograms of genetic spatial autocorrelation results across different spatial scales, for females and males. Distance classes vary from 2km to 100m; panel 4 shows a multiple distance class analysis, with the first distance class increasing from 100m to 1000m for females and males; panel 5 displays the distribution of the maximum distance travelled by recaptured pale field-rats, within and between sessions. Spatial autocorrelation estimates are bounded by 95% bootstrap confidence intervals.

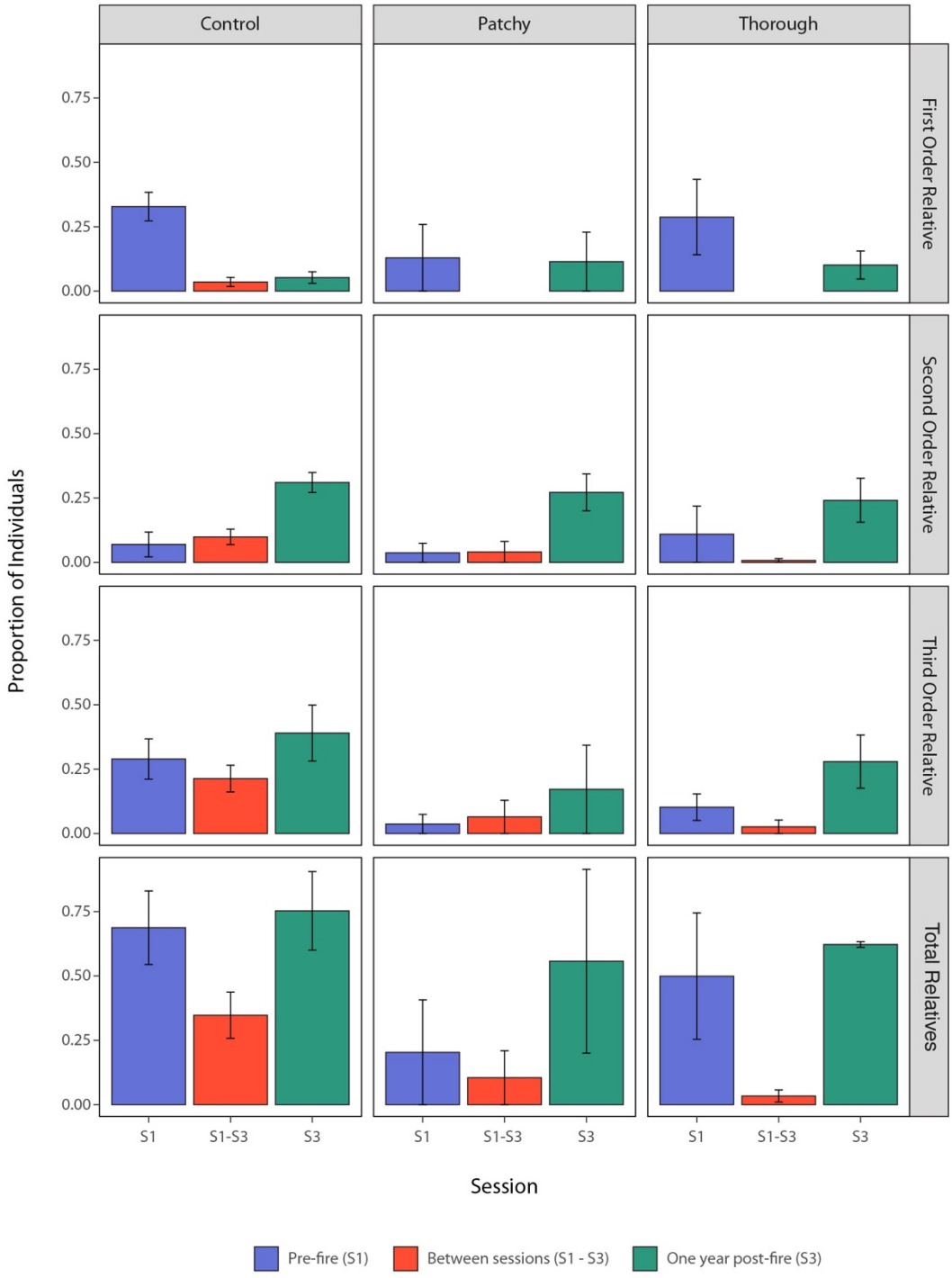

Figure 5. The mean proportion of individuals with a first-, second- or third-order relative across control, patchy and thorough sites (and the total across all relatedness categories), as estimated through parentage analysis. The proportion individuals with a relative was determined within each session (before the fire-experiment and one year after fire) and between these sessions. Error bars represent the standard error of the mean.

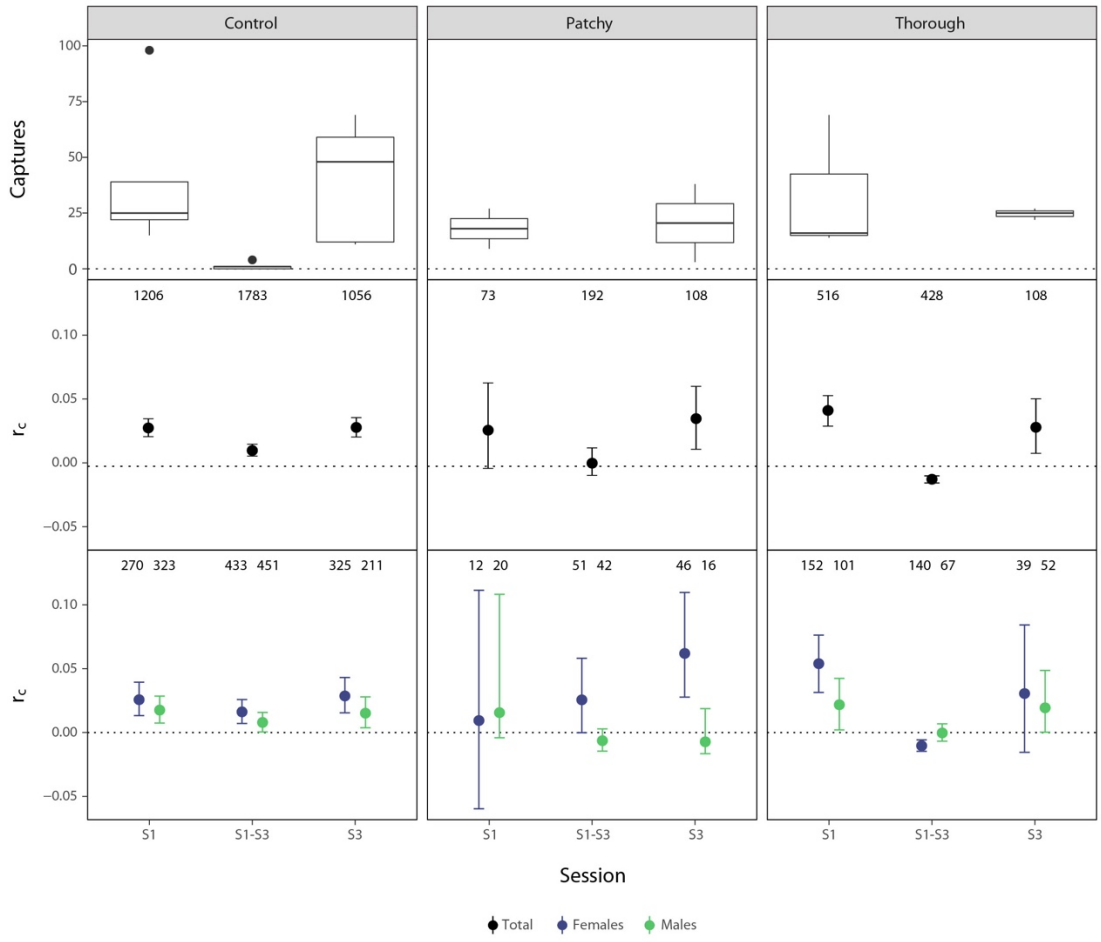

Figure 6. Pale field-rat capture patterns (total) and genetic spatial autocorrelation patterns (total and by sex) before the fire experiment (S1), one year after fire (S3) and between these sessions (S1-S3), over control, patchy and thorough sites. Genetic spatial autocorrelation was measured over a 100m distance class and is bounded by 95% bootstrap confidence intervals. Numbers above estimates represent the number of pairwise comparisons used to calculate r_c.

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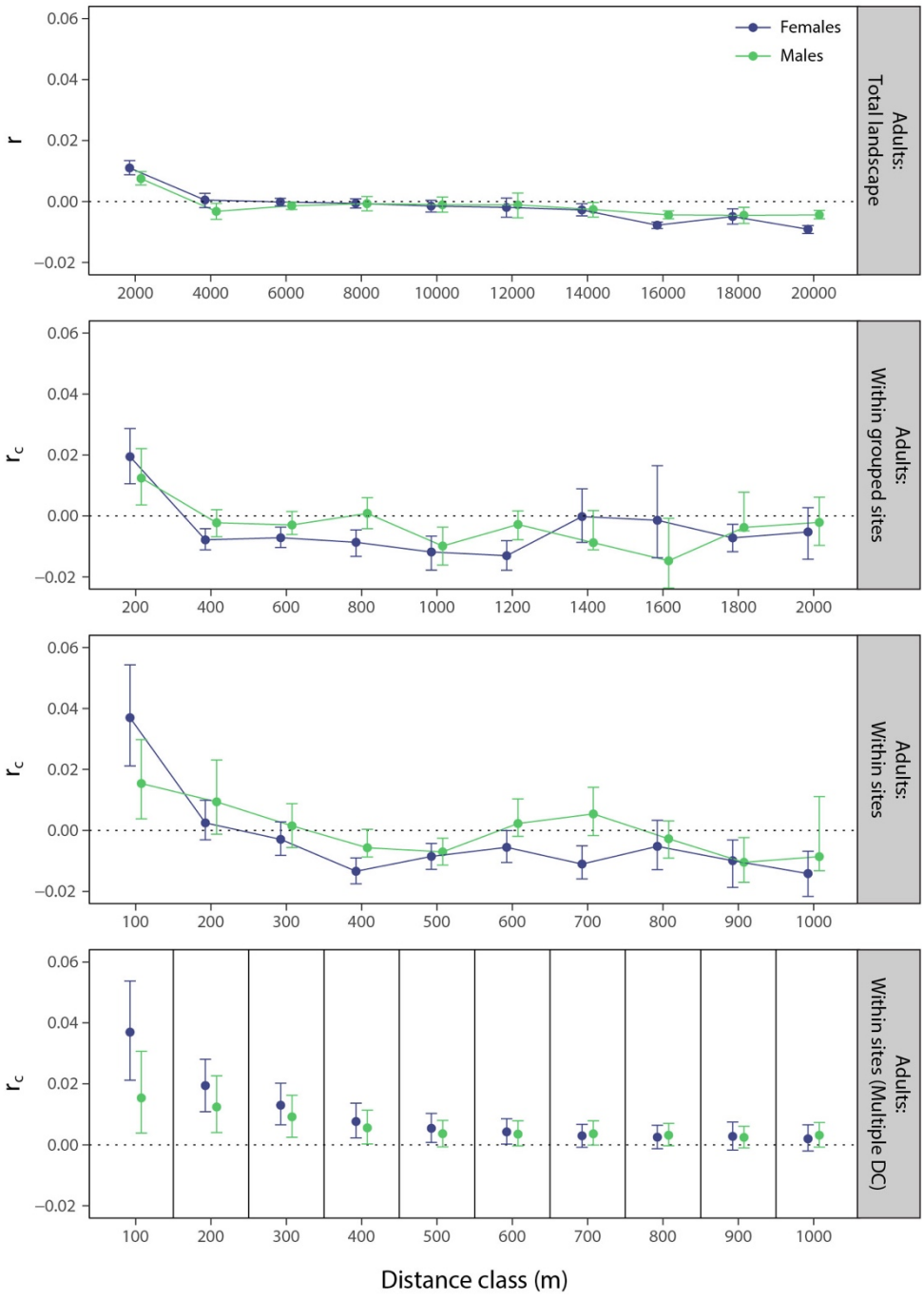
Appendix

Appendix S1. Pairwise F_{ST} patterns before fire (S1), one year after fire (S3) and between sessions (S1-S3).

Session	Site 1	Site 2	N (site 1)	N (site 2)	F_{ST}	p
S1	RS08	RS09	39	27	0.010	0.011
	RS10	RS11	24	9	0.005	0.153
	RS12	RS13	14	13	0.013	0.067
	RS12	RS17	14	15	0.006	0.152
	RS13	RS17	13	15	0.018	0.031
	RS14	RS15	22	67	0.008	0.015
S3	RS08	RS09	57	35	0.007	0.013
	RS10	RS11	12	10	0.013	0.075
	RS12	RS13	27	25	0.010	0.026
	RS12	RS17	27	11	0.008	0.110
	RS13	RS17	25	11	0.005	0.153
	RS14	RS15	48	22	0.008	0.028
S1-S3	RS08	RS08	39	57	0.003	0.055
	RS08	RS09	39	35	0.008	0.007
	RS09	RS08	27	57	0.010	0.009
	RS09	RS09	27	35	0.010	0.012
	RS10	RS10	24	12	0.004	0.201
	RS10	RS11	24	10	0.009	0.060
	RS11	RS10	9	12	0.007	0.164
	RS11	RS11	9	10	0.000	0.405
	RS12	RS12	14	27	0.006	0.106
	RS12	RS13	14	25	0.004	0.135
	RS12	RS17	14	11	0.003	0.217
	RS13	RS12	13	27	0.021	0.009
	RS13	RS13	13	25	0.017	0.021
	RS13	RS17	13	11	0.017	0.038
	RS14	RS14	22	48	0.005	0.074
	RS14	RS15	22	22	0.003	0.177
	RS15	RS14	67	48	0.009	0.003
	RS15	RS15	67	22	0.007	0.035
	RS16	RS16	94	66	0.003	0.038
	RS17	RS12	15	27	0.009	0.042
	RS17	RS13	15	25	0.008	0.056
	RS17	RS17	15	11	0.003	0.223



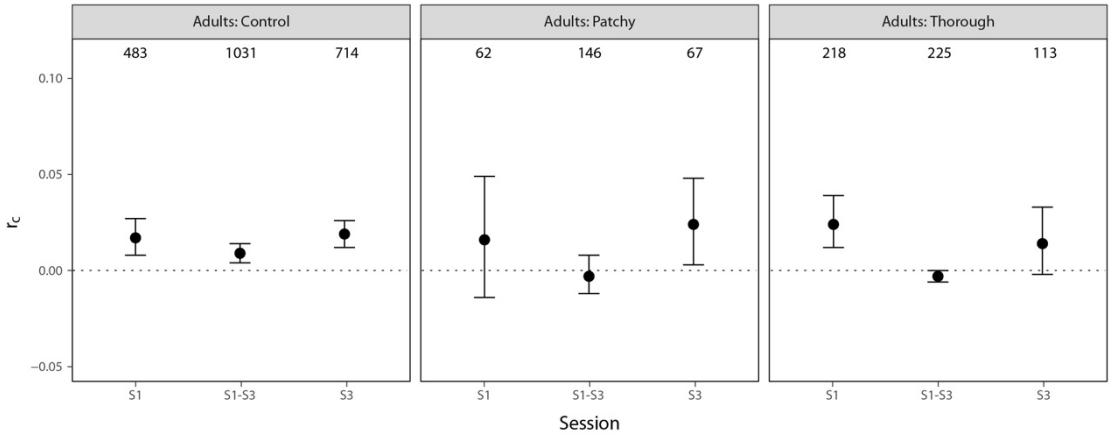
Appendix S2. Correlograms displaying genetic spatial autocorrelation results across different spatial-scales and a multiple distance class analysis, for adult females and males (with juveniles removed from the analyses). Distance classes vary from 100m to 2km. Spatial autocorrelation estimates are bounded by 95% bootstrap confidence intervals.

Appendix S3. Model summaries for the GLMMs investigating the effect of session (session 1- S1 and session 3- S3) and treatment (control, patchy, thorough) on the observed heterozygosity (H_O), expected heterozygosity (H_E) and inbreeding coefficient (*F)* in pale field-rat populations.

Statistic	Variable	Estimate	Std. Error	Z value	p
H_O	Control (Intercept)	0.236	0.003	78.210	**<0.001**
	Patchy	-0.003	0.006	-0.540	0.590
	Thorough	-0.002	0.005	-0.320	0.750
	S3	-0.002	0.004	-0.430	0.670
	Patchy x S3	0.006	0.008	0.700	0.480
	Thorough x S3	0.005	0.007	0.730	0.470
	Random term	Variance	Standard Deviation		
	Group	1.077E-07	3.281 E-04		
H_E	Control (Intercept)	0.267	0.003	81.480	**<0.001**
	Patchy	-0.005	0.006	-0.800	0.420
	Thorough	-0.005	0.005	-0.880	0.380
	S3	-0.002	0.004	-0.500	0.620
	Patchy x S3	0.003	0.008	0.340	0.730
	Thorough x S3	0.007	0.007	0.950	0.340
	Random term	Variance	Standard Deviation		
	Group	8.41E-06	0.003		
F	Control (Intercept)	0.102	0.010	9.990	**<0.001**
	Patchy	-0.013	0.011	-1.220	0.220
	Thorough	0.002	0.010	0.180	0.860
	S3	-0.001	0.007	-0.140	0.890
	Patchy x S3	-0.007	0.014	-0.520	0.600
	Thorough x S3	0.004	0.012	0.330	0.740
	Random term	Variance	Standard Deviation		
	Group	3.765E-05	0.019		

Appendix S4. Pale field-rat scaled [0,1] within site (by session) diversity values (α'). Sites represent trapping locations and treatment and sessions represent trapping periods over time (before the fire experiment- S1, and one-year after fire- S3). Statistical significance was evaluated using Bartlett's test for homogeneity.

Site	Treatment	Group	S1 α'	S3 α'	Mean α'	p
RS08	Control	1	0.286	0.288	0.288	0.953
RS10	Control	2	0.281	0.282	0.282	0.728
RS17	Control	3	0.283	0.283	0.285	0.934
RS14	Control	4	0.286	0.284	0.286	0.706
RS09	Patchy	1	0.285	0.287	0.288	0.975
RS11	Patchy	2	0.287	0.282	0.286	0.855
RS12-13	Thorough	3	0.284	0.285	0.286	0.953
RS15	Thorough	4	0.283	0.285	0.285	0.681



Appendix S5. Genetic spatial autocorrelation patterns before the fire experiment (S1), one-year after fire (S3) and between these sessions (S1-S3), over control, patchy and thorough sites for adult pale field-rats (with juveniles removed from the analysis). Genetic spatial autocorrelation was measured over a 100 m distance class and is bounded by 95% bootstrap confidence intervals. Numbers above estimates represent the number of pairwise comparisons used to calculate r_c.

Chapter 6

Synthesis

Dispersal and reproduction shape social interactions, population dynamics, the spatial layout of individual genotypes, and ultimately the distribution and evolution of species. Understanding these fundamental processes is vital if we are to gain insight into the workings of populations. Furthermore, demographic information can help us to understand the post-fire recovery process. Incorporating this information into fire management strategies aimed at conserving biodiversity will be critical for facilitating population recovery in vulnerable species.

Chapter 2

As the first part of my PhD, in Chapter 2, I used individual-level simulations to investigate the effects of dispersal and mating systems on fine-scale genetic structure at autosomal, mitochondrial and Y chromosome markers. I found that dispersal was the major driver of fine-scale genetic structure across maternally, paternally and biparentally inherited markers. However, when dispersal was restricted, the mating system influenced fine-scale genetic structure differently at the paternally inherited Y chromosome compared to maternally inherited mitochondrial markers. Thus, comparing these patterns between females and males, across these marker types may provide us with a better understanding of the demographic processes occurring within animal populations.

Chapter 3

Empirical research focused on pale field-rat populations in the Kimberley region of Western Australia formed the major component of my PhD. My ultimate goal was to combine direct field observations with genetic analysis to investigate the demographic patterns, fire response, and post-fire recovery in this species. For my genetic analysis, I took advantage of exciting new opportunities to use 1000's of autosomal SNPs (Single Nucleotide Polymorphisms) as genetic markers. However, informed decisions must be made about the bioinformatic pipeline and filtering parameters chosen when using this type of data.

In Chapter 3, I explored an empirical pale field-rat dataset, comprising a range of different marker types, including autosomal SNPs (aligned using different bioinformatic approaches). I found that both bioinformatic pipelines and filtering can impact fine-scale

population genetic results. Aided by additional autosomal microsatellites and by mtDNA and Y chromosome markers with different modes of inheritance for support, I identified key filters that returned the best possible set of high confidence genotypes. My findings highlight the importance of carefully considering how different bioinformatic processes might impact downstream results.

Chapter 4

In Chapter 4, I conducted a fire experiment to explore fire response and habitat preferences in pale field-rat populations, and to make predictions about the post-fire recovery process. I found that pale field-rat populations were severely influenced by fire, with capture and recapture rates significantly declining with increasing fire extent. Pale field-rats showed strong preferences for habitat associated with both dense cover and proximity to watercourses, and these preferences did not change after fire. While pale field-rat populations had completely recovered one year later, my findings suggested that recovery processes may differ depending on the spatial extent of the experimental fires. I predicted that recovery was driven by *in situ* survivors within unburnt refuges after patchy fires, compared to recolonisation from outside the burnt area after thorough fires.

Chapter 5

Finally, in Chapter 5, I test these two alternative extremes of the recovery continuum proposed in Chapter 4: *in situ* survival and recolonisation (with recolonisation stemming from multiple sources or from a single source). I explored how genetic patterns changed from before the fire experiment, to one year after fires of differing spatial scales and intensity. Using a combination of genetic and demographic evidence, my findings suggest that *in situ* survival drives population recovery after patchy fires, compared to recolonisation after thorough fires. Furthermore, these patterns appear to be primarily driven by females, with their dispersal extent potentially increasing with increased fire extent.

The power of combination

My thesis highlights how combining a number of different approaches can be a powerful way to tease apart the complex processes occurring within and among animal

populations. Furthermore, using a combination of approaches can provide novel insights and unexpected results that would not have been detected using one method alone. For example, by combining demographic (patterns of abundance) and genetic data (patterns of relatedness and polymorphic loci), I was able to show that post-fire recovery mechanisms differed between patchy and thorough fires in pale field-rat populations. Demographic evidence also suggested that source populations may be restricted to creek lines and other moist, productive areas. Thus, the pathways from which recolonisation of previously burnt habitat can occur may also be limited.

Of particular interest was the genetic findings which suggested that female (rather than male) dispersal patterns changed after extensive fires, when compared to lower intensity, patchy fires. These unexpected findings indicated that the females in this species may exhibit context dependent 'dispersal polymorphism'. This hypothesis is also consistent with the surprising lack of any strong fine-scale genetic evidence for male-biased dispersal. These results are in stark contrast with several other genetic studies of other small Australian mammals, including bush rats, which have uncovered very strong genetic signatures of male-biased dispersal with genetic analysis over a similar size and scale as in the present study (Peakall et al. 2003). Thus, combining direct field observations, capture-mark-recapture and genetic analyses has provided a more holistic view of the population recovery process and yielded new insights into the sex-specific dispersal patterns in this species.

Finding congruent answers across a combination of approaches has also been an important outcome of my research. This has helped to reinforce the evidence for specific biological and ecological predictions. For example, in Chapter 3, combining a range of molecular markers allowed me to confirm the robustness of genetic results based on SNP panels that were genotyped using different bioinformatic pipelines and filtered for different criteria. Congruent patterns between the demographic and genetic results also provided multiple lines of evidence for the two different female-specific recovery processes uncovered in Chapter 5.

Finally, different perspectives can be gained by combining multiple approaches. For example, in Chapter 2, I used a combination of molecular markers with different modes of inheritance to investigate dispersal and mating systems. By using maternally inherited mitochondrial markers in combination with the paternally inherited Y chromosome, I was able to gain a sex-specific perspective on gene flow in animal populations. These markers showed differing levels of fine-scale genetic structure between the sexes, revealing variation in dispersal, reproductive skew and multiple mating.

Mammal declines in northern Australia

We currently lack a thorough understanding of the mechanisms underlying population recovery in small mammals and other vertebrates (Driscoll et al. 2010, Griffiths and Brook 2014, Hossack and Honeycutt 2017). However, increasingly it appears that *in situ* survival due to habitat heterogeneity and fire patchiness is an important driver of post-fire population recovery for many species across a broad range of habitats (Schwilk and Keeley 1998, Hochkirch and Adorf 2007, Watson et al. 2012, Leahy et al. 2016, Banks et al. 2017, Hossack and Honeycutt 2017). In fact, in intact ecosystems, vertebrate populations appear to be fairly resilient to wildfire (Hossack and Honeycutt 2017). However, human induced change through direct and indirect factors such as climate change, the spread of exotic grasses and changes in precipitation have increased the extent and frequency of severe wildfires across a range of ecosystems (Gill and Allan 2008, Bowman et al. 2009, Cansler and McKenzie 2014, Griffiths and Brook 2014). These large, homogeneous fires leave few survivors, and the dispersal capability of many animals may be inadequate to facilitate population recovery over these scales (Banks et al. 2017). Similarly, species that rely on food resources that only become available several years after fire are compromised by failure to retain sufficient areas of long unburnt vegetation (Atchison 2009, Legge et al. 2015). Therefore, understanding how post-fire recovery mechanisms change with fire extent is an increasingly relevant question across many ecosystems, and incorporating this information into fire management strategies aimed at conserving biodiversity will be vital.

Fires in northern Australia can burn for months over many thousands of square kilometres (Russell-Smith et al. 2003, Radford 2010). My findings support a growing body

of research suggesting that these frequent, large, homogeneous fires are removing small mammals from the landscape (Andersen et al. 2005, Legge et al. 2008, Woinarski et al. 2011, Lawes et al. 2015). My research suggests that the primary mechanism driving recovery after thorough fires (i.e. wildfires) is likely to be recolonisation, due to low survival in intensely burnt areas. Furthermore, despite the often apparent uniformity of savanna landscapes (to human eyes), recolonisation pathways are nonetheless likely to be restricted. For example, while constraints on animal movement include the more obvious major landscape features such as rocky outcrops, sand seeps and water courses, even more subtle habitat attributes, such as terrain and substrate can restrict animal movements (Woinarski et al. 2005).

The additive effects of fire, predation by feral cats and grazing by introduced herbivores likely makes the post-fire landscape unfavourable for recolonisation and dispersal for pale field-rats and other vulnerable mammal species. Therefore, recolonisation will be a slow process if fires occur over a scale larger than which pale field-rats perceive their surroundings, or are capable of moving over several generations (Clarke 2008, Mutz et al. 2017). Restrictions to specific dispersal routes, such as along water courses, would exacerbate this effect, particularly in combination with the continued loss and degradation of riparian habitat due to grazing (Skroblin and Legge 2012, 2013).

If fires occur over a timescale faster than populations can recover through recolonisation, a regime of frequent wildfire may quickly remove any source of individuals in the landscape from which recolonisation can occur, leading to local extinction over a scale of 10's to 100's of square km (McGregor et al. 2016, Mutz et al. 2017). Furthermore, intensive burning over large areas may also lead to a temporary increase feral cat populations, due to increased hunting success in these environments (McGregor et al. 2014, 2016). Amplified predation would further increase pressure on vulnerable small mammals at the regional scale. Thus, impeded post-fire recovery may also be a strong contributing factor in the widespread decline of small-mammals across northern Australia.

Future directions: fire management

For my PhD research, it was not feasible to carry out a fire experiment over the scale more representative of a late dry season, unmanaged fire (100's of square km). Thus, it was not possible to determine the maximum distance pale field-rats are able to move to recolonise previously burnt habitat. However, movements detected through capture-mark-recapture were mostly over the scale of tens of metres (with a maximum of 1882 m). This distance also aligns with the extent of fine-scale genetic structure detected in this system. This suggests that, in order to facilitate post-fire recovery, fire management strategies should aim to maintain source populations within at most 2 to 3 km of the fire-scar. Furthermore, fire management strategies with the objective of increasing fine-grained patchiness within burnt areas (over a scale of hundreds of meters) will not only be beneficial for mitigating the immediate effects of fire on small mammal populations, but will likely also facilitate the recovery of vulnerable small mammal species through *in situ* survival within unburnt refuges.

Future research: landscape genetics

The apparent reliance of pale field-rats on habitat closely associated with watercourses warrants further investigation. During my PhD, I utilised fine-scale genetic analyses to understand dispersal patterns in pale field-rat populations. However, landscape genetics could be a particularly valuable addition to the present study. This approach combines multiple methods from landscape ecology, spatial statistics and population genetics to understand how genetic variation is distributed across the landscape (Storfer et al. 2010). A landscape genetics approach could allow us to more definitively characterise dispersal routes across the landscape for this species. Thus, fire management could potentially use this as a tool when planning prescribed fires, by ensuring burnt areas are connected to suitable source populations.

Landscape genetics is an important tool in conservation and has already been utilised to understand gene flow and landscape connectivity in northern quolls, a carnivorous marsupial that has also experienced marked declines in northern Australia. Hohnen et al. (2016) found that variation in patterns of genetic structure across populations of this species was likely driven by rain fall and terrain ruggedness. They

suggested that populations connected by areas that were topographically less complex may have been historically connected. Thus, threat management in open habitat may increase population connectivity in this threatened species.

Final conclusions

During my PhD research, I have used a combination of field-based experiments computer simulations, and molecular population genetic techniques to explore how biological and ecological processes shape populations and their underlying genetic diversity. My research highlights the benefits of using a combined approach, revealing novel insights into the demographic processes occurring within populations, the response of small-mammal populations to fire, and the post-fire recovery process. A continued focus on the fine-scale mechanisms underlying population dynamics, which until now have been largely neglected, will be an important addition to the body of research aiming to understand and halt the widespread decline of Australia's unique mammal fauna.

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